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VOLATILE NITROGEN LOSSES FROM SOME ALBERTA SOILS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "VOLATILE NITROGEN LOSSES FROM SOME ALBERTA SOILS" submitted by Mohammad Fasahat Ali Khan, B.Sc.(Agri.), M.Sc.(Agri.), in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

This study was undertaken to find out the extent of volatile losses of nitrogen from Alberta soils and the effect of plants on such losses. Nitrogen can be applied to a soil in various forms, and in this study ammonium sulphate, ammonium nitrate, sodium nitrate, ammonium phosphate, urea and dried blood were used as the nitrogen carriers. Greenhouse experiments were carried out with six soils from five soil zones of Alberta, namely Grey Wooded, Black, Brown, Dark Brown, and Dark Grey Wooded, and one Organic (Peat) soil. These soils were treated with different carriers and kept moist at field capacity and nitrogen losses were determined by comparing the total nitrogen in 'non-treated' with that in 'treated' soils. It was found that large losses of added nitrogen took place in Grey Wooded, Black (Solonetzic), Brown, and Dark Brown soils of Alberta. The effect of plants on the loss was tried on Grey Wooded and Black (Chernozemic) soils. The presence of plants caused a significant decrease in nitrogen loss from Grey Wooded soil when ammonium sulphate was the nitrogen carrier, and where nitrogen taken up by the plants was not considered as a loss from the system.

The inherent ability of these soils to denitrify was determined by using the Warburg apparatus. Soil samples with known amounts of nitrate and glucose were incubated anaerobically and denitrification measured. Two parameters were determined: Denitrifying Capacity (D.C.), defined as the per-

centage of added nitrate denitrified, and Denitrifying Potential (D.P.), the amount of nitrogen gas evolved per unit weight of soil per unit time. The surface soils from cultivated fields, the same soils that were used in the greenhouse, were found to have high D.C. and D.P. values, but these could not be related to nitrogen loss found under greenhouse conditions. Examination of the parameters for soils from the various horizons of seven profiles showed that denitrification takes place in soils from lower horizons but at a slower rate than in soil of upper horizons.

Denitrification studies on soil from rotation plots showed that the results obtained could not be related to rotation and fertilizer practices. However, low D.C. and D.P. values were found in soil samples from Beaverlodge as a group as compared to other soils in Alberta. Comparison of surface samples from cultivated and virgin land showed that cultivated field samples had low D.C. and D.P. values, showing that cultivation somehow changed either the number and type of denitrifiers or other environmental factors.

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INTRODUCTION

Nitrogen may be lost from the soil in various ways. For example, nitrogen uptake by plants may be considered a loss from the soil if the plants are subsequently removed, as is the case with many agricultural crops. A similar process is the assimilation of nitrogen compounds by soil microorganisms, which is, however, only a temporary loss since the nitrogen in the microbial cells is returned to the soil upon the death of the organisms. Soluble forms of nitrogen may also be leached from the soil in areas of high rainfall. In recent years, losses of nitrogen in gaseous form, due to denitrification, have been shown to be of considerable agronomic importance. Volatile nitrogen losses are permanent losses and in view of this element being the limiting factor for the growth of crop plants in many soils, the study of the process of denitrification assumes considerable significance.

Denitrification is a microbiological process in which oxidized forms of nitrogen like nitrate and nitrite are reduced to elemental nitrogen (N_2), oxides of nitrogen, chiefly nitrous oxide (N_2O) and occasionally nitric oxide (NO). The microorganisms involved, commonly referred to as denitrifiers, are aerobic bacteria that have the unique ability to use oxidized nitrogen in the absence of oxygen as the terminal electron acceptor in respiration. The denitrifiers occur widely in soils and are definitely a part of the normal micro-flora.

Since the denitrifying organisms reduce nitrates under

anaerobic conditions only, denitrification has not generally been considered to be of much importance in normal, well-aerated soils. However, recent findings have indicated that even under normal conditions, denitrification may be going on because of local anaerobic conditions in the pore spaces of the soils.

Agronomists in Alberta are recommending progressively higher rates of nitrogenous fertilizers as either nitrate or ammonium salts. The latter is usually converted to nitrate by the nitrifiers in soil and taken up by plants in this form. It is probable that a part of this added nitrogen is used by the denitrifying microorganisms and lost as gas. Cultivation also disturbs nitrogen equilibrium in soils. When new land is broken, the nitrogen status of the soil falls rapidly until a new equilibrium is reached. In some soils, such rapid losses cannot be accounted for by the nitrogen utilized by crop plants or leaching out of nitrates in drainage water. Numerous laboratory and greenhouse experiments and data from lysimeters have shown that mineral nitrogen does not remain in soil in that form for long; it is either taken up by higher plants and other living organisms, leached out of the soil or lost by volatilization. Thus, it is most likely that denitrification may be involved in nitrogen losses from broken prairie soils.

The direct study of denitrification under field or greenhouse conditions is not possible because the measurement of changes in gaseous nitrogen or oxides of nitrogen cannot be carried out, and also because the differences between initial and final total nitrogen content of any soil over a certain period of time is not a valid measure of denitrification losses

because nitrogen fixation may be going on simultaneously with denitrification.

Since denitrification is an anaerobic process and denitrifiers are fully active in the presence of a sufficient supply of oxidizable carbon, the inherent ability of soils to denitrify can be compared by providing suitable conditions and measuring the amount of nitrogen lost by several methods. One such method is a modification of Olsen's method of total nitrogen analysis, another by direct manometric measurement of N_2 in a Warburg apparatus, or by using labelled nitrogen. The ability of a soil to transform added NO_3^--N to nitrogenous gas which escapes, has been termed "denitrifying capacity" and has been defined as the percentage of added nitrate-nitrogen lost as nitrogen gas in the presence of an adequate carbon source under anaerobic conditions. The rate of nitrogen gas evolution from soil expressed as µg N_2/g./hr. has been termed "denitrifying potential".

The present study was undertaken with the following objectives:

(1) To determine whether or not volatilization losses of nitrogen occur from Alberta soils following nitrogen fertilization and to determine whether any such losses are influenced by soil type, nature of nitrogen carrier, and the presence of plants.

(2) To determine the "denitrifying capacity and potential" of some Alberta soils, and to determine the influence of soil type, profile depth, length of storage of soil, and agronomic treatments.

LITERATURE REVIEW

Historical[1]

Sir Humphrey Davy in 1814 reported the evolution of nitrogen from soil, but the reduction of nitrate in soil was first demonstrated by Schönbein in 1868. The fact that nitrogen may be lost from soil was also demonstrated indirectly by Boussingault (Russell and Richard, 1917). Boussingault found less nitrogen in soil plus plant at the end of the experiment than in soil plus seed at the beginning. He attributed this difference to loss as nitrogen gas. However, it was not until 1882 that Gayon and Dupetit, and Deherain and Maquenne established that there are bacteria in soil capable of reducing nitrates to nitrogen and oxides of nitrogen. The chemical reaction, whereby carbohydrates or organic acid of the media are decomposed with the formation of CO_2 and nascent hydrogen, which reduces the nitrate was also suggested by these workers. Lawes, Gilbert and Warington pointed out the same year that considerable evolution of nitrogen may take place from soil saturated with water or improperly aerated and receiving heavy applications of manure. Wagner in 1895 (Russell and Richards, 1917) was the first to claim that the mechanism of such loss was solely through a reduction process. He considered that nitrates are always present in manure, and these

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[1] Material in this section is largely from Waksman (1927), who quotes the original papers.

decompose in the absence of air giving rise to nitrogen, thus restating the original idea of Gayon and Dupetit. Immendorf (Russell and Richards, 1917), on the other hand, favored an oxidation hypothesis and visualized the direct oxidation or combustion of nitrogen compounds to nitrogen gas. He was supported in this claim by Pfeiffer and co-workers, as quoted by Russell and Richards (1917). This view was commonly accepted in Germany, and Rumker (1909) "directed attention to the great amount of nitrification that would be required if the whole of the nitrogen were lost by alternate nitrification and denitrification, and asked where the lime is to come from, apparently overlooking the fact that only a small amount is necessary as it could be used over and over again". On the other hand, Müntz and Lainé (1911) supported the reduction hypothesis, on the basis of data obtained with perfusion technique. They found that ammonia added to a percolation filter did not result in the evolution of nitrogen, but when nitrate was added, nitrogen loss was considerable.

That denitrification is predominantly a bacterial process was accepted by the earlier workers. In a classic experiment carried out by Wagner (1895), it was observed that when organic nitrogen and nitrate were added simultaneously to a cultivated field, nitrogen loss was considerable. This led him to conclude that while nitrate acted as a substrate for denitrification, the added organic matter provided the appropriate microorganisms to bring about denitrification. Pfeiffer and Lemmermann (1898) claimed that the observed loss of nitrogen is not real, and in fact, no loss of nitrogen takes place as a

result of addition of manure to soil. The lack of nitrogen often observed was claimed by him to be due to other causes. Lemmermann (1900) found that although nitrates completely disappeared from soil due to reduction, it may appear as ammonia and nitrite by the action of a large number of microorganisms, and these products can again be acted upon by nitrifying bacteria. Moreover, a portion of nitrate is also assimilated by some microorganisms. Thus, according to Lemmermann, as well as some other workers (e.g. Gerlach and Vogel, 1901; Löhnis, 1905), the apparent disappearance of nitrate is due to its being stored away in soil in some other organic forms.

Koch and Pettit (1910) found that denitrification takes place in an entirely different manner in soil than in solution. In solution, in the presence of an organic substrate, the bacteria may liberate practically all the nitrogen present as nitrate; while in moderately moist soil, only protein may be formed from nitrate. But if the soil is very moist, denitrifiers behave as in solution and liberate considerable nitrogen gas. Thus, according to Waksman, the original suggestion of Winogradsky that the introduction of large quantities of manure favors denitrification, and prevents nitrification, has been proved erroneous. Waksman (1927), however, thought that only under special circumstances such as anaerobic conditions provided by submerged soils in tropics or elsewhere, loss of nitrogen due to denitrification may be of some consequence.

Geochemistry

An indirect proof of bacterial reactions in soil

resulting in a continuous supply of nitrous oxide (N_2O) to the atmosphere has been provided by the geochemical data of Goody and Walshaw (1953), when they found that the N_2O content of the atmosphere remained almost constant, although at the same time it was being decomposed in the stratosphere. These workers were of the opinion that bacterial reactions in the soil supply nitrous oxide to the atmosphere at a rate sufficient to compensate photochemical decomposition. Arnold (1954) has considered the geophysical implication of the evolution of nitrous oxide gas from the soil surface. He has quoted Prasolov (1946), who claimed that soil occupies 0.16 of the earth's surface after excluding tundra, desert, etc. Considering the depth of biologically active top soil to be within the range of 10-100 cm., 30 cm. was taken by him as a reasonable value for the mean depth and 1.5 $g./cm.^3$ for its density. The concentration of ammonium and nitrate nitrogen, the potential source of nitrous oxide, although very variable, was taken to lie within the range of 1-100 ppm., and thus 10 ppm. was taken as a conservative average. Based on these figures, he calculated the potential source of gaseous nitrogen compounds over the total earth's surface to be 1.6×10^8 $molecules/cm.^2$. Since Goody and Walshaw (1953), estimated the destruction rate of nitrous oxide in a vertical column of the atmosphere to be approximately 8×10^{10} $molecules/cm.^2$ sec.; in order to achieve a rate of supply to replace this loss, Arnold considered that a reaction with a time constant* of 2.0×10^7 sec., or about 230

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* Time constant defines the initial rate of transformation of the available nitrogen in the soil into nitrogen as N_2O appearing in the atmosphere. Thus if x g. dry soil contain y ppm. of available nitrogen, the time constant is T when $\frac{xy\ 10^{-6}}{T}$ g. nitrogen per day is the initial rate of evolution of the gas.

days is necessary. After careful consideration of the evolution of gas under very wet and fairly wet conditions, he concluded that the periodic wetting of soil surface by rainfall would lead to an expected time constant of the order of 100-1000 days, and, therefore, the soil surface was considered to be the major source of supply of nitrous oxide.

Mechanisms of Nitrogen Volatilization Losses

The fact that addition of nitrogenous material to soils results in some loss of nitrogen by volatilization is accepted by most workers in this field. But the mechanism of such a loss has not been fully established and various workers have proposed different mechanisms depending upon experimental conditions and the types of soil on which they had been working. To sum up, four mechanisms have been proposed, namely:

(a) non-biological losses as ammonia,

(b) chemical decomposition of nitrate under acid conditions to yield nitrogen oxides,

(c) production of nitrogen gas by the non-enzymatic reaction of nitrous acid with ammonia or amino acids, and

(d) microbial denitrification leading to the liberation of nitrogen gas and nitrous oxide.

According to Allison (1955), there is universal agreement that under suitable conditions, e.g. high pH, drying soils high in ammonia content, low exchange capacity, etc., ammonia is rapidly lost from soil by volatilization. Such losses may amount to 25 per cent or more of the ammonia added or formed.

Heck (1931) has shown that the main reason for low recovery of nitrogen in crop under long-time field experiments where manure has been used is the volatilization of ammonia, especially when the manure is not incorporated into the soil immediately after spreading. Volk (1959) found that surface application of urea at the rate of 100 lb. nitrogen per acre to different turfs and bare soils resulted in average volatile losses as ammonia from 21 to 29 per cent compared with 0.3 per cent from ammonium nitrate. Russell (1961) believes that nitrogen can be lost in the form of ammonia only when an ammonium fertilizer is added to the surface of a calcareous soil. It is also possible that such losses occur if readily decomposable organic matter high in nitrogen is added to alkaline soils in hot wet conditions, such as occur in some paddy fields in the tropical countries (Sreenivasan and Subrahmanyan, 1935).

Loss of nitrogen from soils by purely chemical reactions has been proposed on a number of occasions. Nitrous acid can be converted to nitric oxide under acidic conditions and such decomposition is appreciable at a pH of about 5.0 (Allison and Doetsch, 1950), and the rate and extent of decomposition are greatly accelerated with increase in acidity. The nitrogenous compound involved in all the chemical reactions is the nitrous acid which is formed in soil by biological agencies. Nitrous acid is fairly stable at pH values above 5.5 to 6.6, but the rate of breakdown increases greatly with increase in acidity. The reaction involved is:

$$3\ HNO_2 \longrightarrow 2\ NO + HNO_3 + H_2O$$

The NO formed is comparatively stable and may either escape to the air, be absorbed by soil, or react with the oxygen of the air to form the nitrogen oxides NO_2 and N_2O_4. When dissolved in water in the presence of air, the nitrogen oxides form nitrous and nitric acids. Thus, there are various possibilities in the chemical breakdown of nitrous acid. In the case of a number of acid soils of the Netherlands, Gerretsen and de Hoop (1957) reported that losses of up to 74 per cent of the ammonium sulphate added were observed. On further examination of the conditions which promote volatilization of nitrogen, they found that when the initial pH of the medium, both in solution and soil, favors the nitrifying bacteria and the buffer capacity of the medium is of such a magnitude that pH drops below 5.5 during nitrification, volatilization of nitrogen is imminent. They suspected that ammonification and nitrification are linked in some way, because both these processes often coincided. The form in which nitrogen was lost was not only as oxides of nitrogen but to a greater extent as nitrogen gas. In one of their experiments, these workers observed losses of 4.8 mg. NH_4^+-N/100 ml. and 8.4 mg. NO_2^--N/100 ml. when 44.8 mg. NH_4^+-N/100 ml. and 35.8 mg. NO_2^--N/100 ml. were added to a sterile buffer solution of pH 4 and aerated at room temperature for 6 hours. They, therefore, concluded that the observed loss is due to chemical reaction of undissociated nitrous acid with ammonia compounds giving off molecular nitrogen gas.

Smith and Clark (1960) found that the amount of nitrogen evolved was much smaller than claimed by Gerretsen and

de Hoop, and showed that the discrepancy between the two tests is partially explained by the failure to determine nitrate nitrogen. Under the aerobic and acidic conditions employed, it appears highly probable that considerable nitrate would have been formed. In the case of Fort Collins loam, having pH 6.3 and 3.86 per cent organic matter, they found N_2O as one of the products of nitrite decomposition. This soil also favored more nitrogen evolution from a nitrite treatment when ammonium sulphate was omitted than when it was added. Such an observation can best be reconciled by considering that all the nitrogen evolved came from nitrite. These workers, therefore, concluded that it is improbable that a Van Slyke reaction is predominantly responsible for the nitrogen loss. The evolution of N_2 and N_2O and the disappearance of nitrite nitrogen from the substrate without diminution of ammonium nitrogen strongly suggests that nitrite instability is involved in the loss of nitrogen from acidic and aerobic soil. Clark, Beard and Smith (1960) investigated the extent to which arable field soils differ in their capacity to transform or to accumulate nitrite and the extent to which any intermediate nitrite accumulation in soil influences the recovery of applied mineral nitrogen. On the basis of their tests, which included aerobic incubation of various soil samples with urea and nitrite and the analyses of mineral nitrogen recovery including NH_4^+-N, NO_2^--N and NO_3^--N and determination of pH, they thought it highly probable that HNO_2 instability or reactivity is involved, rather than NH_3 -volatilization or enzymatic denitrification in well-aerated soils.

Madhok and Uddin (1946) have however reported that nitrous acid may be lost from soils with pH above 7 on desiccation, although the data presented is rather insufficient to throw any light on the mechanism of such a loss. Among the factors found by them to be responsible for the losses were CO_2 and NH_3 of the air, exchangeable hydrogen of soil, and catalytic action of some soil constituents. Nitrites can react with amino acids and ammonia to form nitrogen gas by means of the well known Van Slyke reaction. On the basis of studies made by Allison and Doetsch (1950) and Allison, Doetsch and Sterling (1952) on this mechanism, the possibility of losses in soils in this manner is rather remote. One of the main reasons is the fact that nitrous acid is very unstable, especially in acid soil conditions, and therefore it may decompose to NO before it can react with amino acids or ammonia. Moreover, at the pH values at which the Van Slyke reaction can occur, the conditions for biological production of nitrous acid is most unfavorable. Wahhab and Uddin (1954) claimed that in soils of Punjab (Pakistan) ammonium and nitrite ions can interact only on desiccation and cause significant loss of nitrogen. These soils have a pH ranging between 8.0 and 8.3. With low concentrations of these added ions, because of magnitude of experimental error in estimating NH_4^+-N and NO_2^--N in soil, no significant loss due to interaction could be obtained. Also, when desiccation was prevented and the time of contact of the reacting ions was increased, no loss occurred even with higher concentration of these ions, showing that they failed to interact. They also noted that loss due to interaction of NH_4^+ and NO_2^- ions on

desiccation was higher in the case of sandy soils than in the case of loams. The losses of nitrogen, on desiccation, from alkaline soils due to volatilization of NH_3 from $(NH_4)_2SO_4$ and to spontaneous decomposition of $NaNO_2$ were of much greater magnitude than those lost through the interaction of NH_4^+ and NO_2^- ions when the soil reaction was not very alkaline; but the loss of NO_2^--N due to interaction suddenly rose in highly alkaline soils. Wahhab and Uddin (1955) further showed that light has a profound effect on the loss of nitrogen causing an interaction between NH_4^+ and NO_2^- ions. Their data show slight losses of nitrogen when $NaNO_2$ solution alone is exposed to the sun. The loss of nitrogen is significant when $NaNO_2$ and $(NH_4)_2SO_4$ are mixed and exposed to the sun, the loss being increased about 42 per cent by such exposure. Soil was found by them to have no catalytic influence during photodenitrification. Increased loss of nitrogen in the presence of soil was found to be due to changes in pH of the medium. When pH values were adjusted to the same value, the presence of soil made no significant difference in the loss.

Allison (1955) has discussed the above results of Wahhab and Uddin and claimed that under the condition of the experiment, the interaction referred to by them is not the same as evidenced in the Van Slyke reaction at pH values below 7. He argued that the Van Slyke reaction can occur only where free nitrous acid (not nitrite ion) is present. The loss reported by Wahhab and Uddin must, therefore, have been brought about by another mechanism, probably the formation of ammonium nitrite from the rearrangement of the ions of the added salt.

Ammonium nitrite decomposes readily to nitrogen gas, water and sometimes traces of ammonia, especially at temperatures of 50°C and above. Allison, however, did not think the losses of this type can be of much practical importance under normal field conditions, because the concentrations of NO_2^- and NH_4^+ are too low for much of the nitrogen to be volatilized.

Allison (1963) summarized some of the accumulated results on the aspect of nitrogen losses through nitrous acid or nitrites and concluded that the gaseous losses of nitrogen from soils may well be greater via ammonium nitrite decomposition than by any other chemical mechanism proposed. Ammonium nitrite is stable in dilute solution, except at low pH value, but in soils the chances of loss of nitrogen would be considerably increased by concentration of the soil solution during drying. On the other hand, sorption of the ammonia or ammonium ions by the soil would decrease the opportunities. Nitrous acid may react with substances other than ammonia. Recently Stevenson and Swaby (1963) and Stevenson and Kirkham (1964) identified methyl nitrite, a nitrogen gas obtained by the action of nitrous acid with lignin. With regard to such observations, Allison thought that ammonium nitrite may be an intermediate in such reactions, although it remains to be determined. In case this pathway of loss is operative in soils, which is most likely, but certainly not proven, Allison thought that much of the loss that is often attributed to so-called aerobic denitrification may be as a result of the formation and decomposition of ammonium nitrite. Ammonium nitrite decomposition can proceed slowly under a variety of soil conditions and therefore a large loss is not dependent on a speedy reaction. In such a

situation, loss of nitrogen can be reduced by making the soil conditions favorable to rapid nitrate formation, and applying fertilizer nitrogen at such times and in such amounts that crops can assimilate it rapidly.

Microbial Denitrification

It seems to be generally agreed among soil bacteriologists that although losses occur by chemical reactions, most of the nitrogen lost from soils, other than as ammonia, is brought about by denitrifying bacteria (Allison, 1955).

The denitrifying bacteria, so far investigated, belong to the genera *Pseudomonas*, *Achromobacter*, *Bacillus* and *Micrococcus*. There are even some autotrophs such as *Thiobacillus denitrificans* which also carry on denitrification with concomitant oxidation of sulphur (Baalsrud and Baalsrud, 1954). In a recent study, *Pseudomonas* and *Achromobacter* were found to be the dominant genera in soil and *Bacillus* species, though abundant as a result of persistence of endospores, were thought to be unimportant (Valera and Alexander, 1961).

It is difficult to cite many physiological properties which are characteristic only of denitrifiers and are related to the denitrification process (Delwiche, 1956). However, the relationship of oxygen to denitrification and the effect of pH on the reaction are of particular interest. Concerning the aerobic and anaerobic nature of the reaction, there is much controversy in the literature. It appears that much of the difficulty which has arisen in this regard has its origin in the selection of experimental conditions. As pointed out by

Delwiche (1956), heavy suspension of cells, even though well shaken in manometric vessels with an atmosphere of oxygen, will probably utilize oxygen at such a rate, substrate permitting, as to lower the oxygen tension appreciably in certain areas of the vessel. Skerman, Lack and Millis (1951) found that unadapted cells of Pseudomonas denitrificans did not apparently reduce the added nitrate while oxygen was present in the media. Previously, Meiklejohn (1940) found that her strains of Pseudomonas sp. could denitrify in aerated as well as in anaerobic cultures. She also examined the counts of denitrifying population and found that the number of bacteria increased with increased aeration. Since she found that a greater amount of nitrogen was retained in aerated cultures, and also because "protein" nitrogen was found to be directly proportional to bacterial number, she concluded that the observation of increased loss of nitrogen from anaerobic cultures does not signify a greater 'denitrifying power'. On the contrary, the large number of bacteria in aerated cultures lock up more nitrogen in their cell protein.

Skerman and MacRae (1957) on the basis of their studies with P. denitrificans did not agree with the findings of Meiklejohn on the grounds that inadequate aeration was responsible for such findings. They therefore claimed that no nitrate reduction can take place when oxygen is continually present even at very low concentration, and this failure of the cells to use nitrate in the presence of oxygen was most likely due to competition between oxygen and nitratase for the donor electron. It may be mentioned here that present

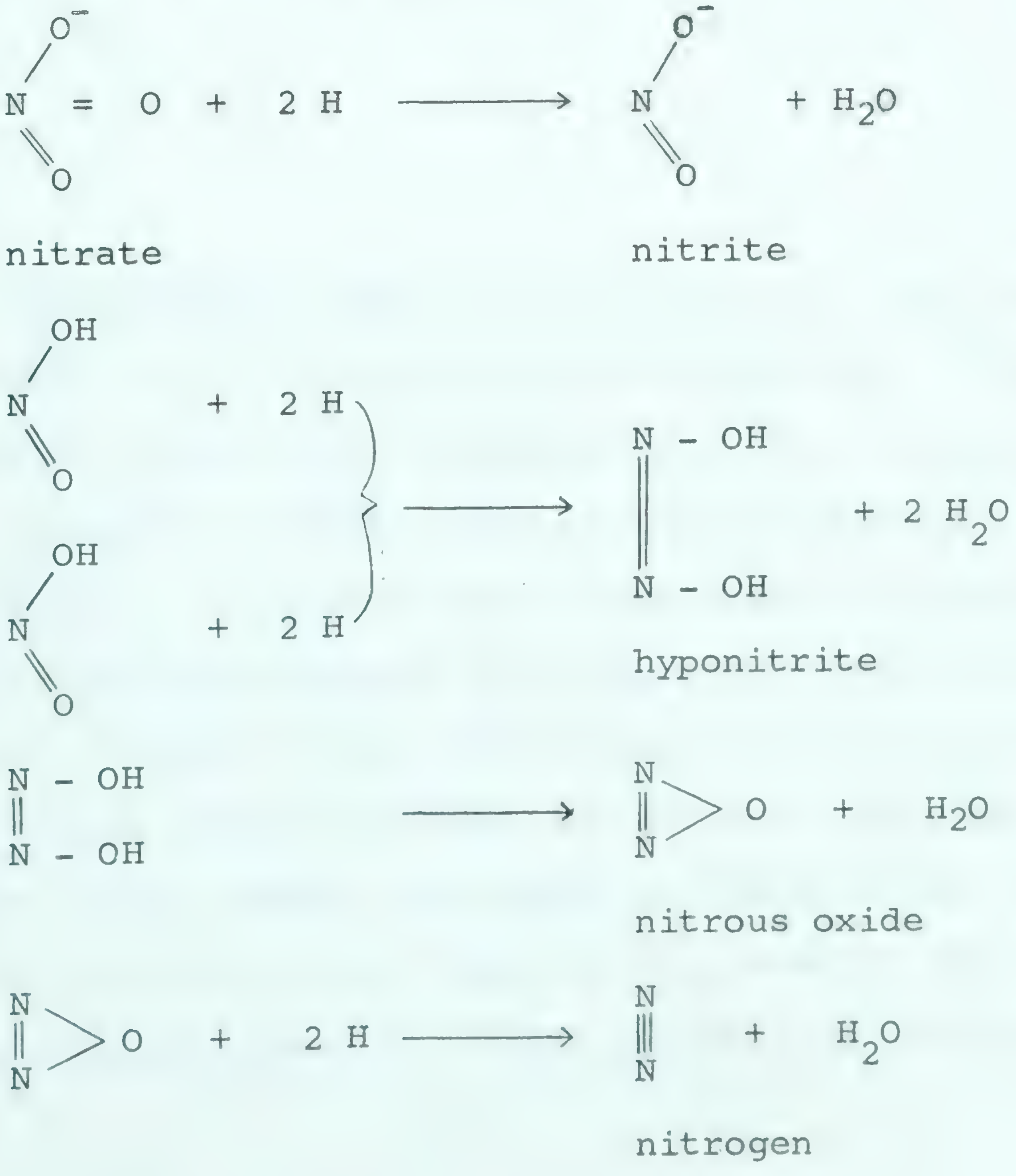
workers in this field believe that oxygen prevents nitratase activity as well as production of the enzyme by the denitrifier. Thus nitrate reduction can occur only when the available oxygen fails to satisfy the demand and nitrate can effectively compete as hydrogen acceptor in a system which is primarily geared to aerobic oxidation.

The pH of the medium has a profound effect on denitrification by bacterial cultures. The optimum pH for most denitrifiers has been found between 7.0 and 8.0. Karlsen (1938, as quoted by Delwiche, 1956) found that P. aeruginosa would denitrify at pH values ranging from 5.8 to 9.2 with an optimal value between 7.0 and 8.0. Since active denitrification by a culture may cause the rise of the pH of the medium, the buffering capacity of the medium exerts a strong influence on the denitrification by the culture in that medium. pH has also been found to have an influence on gaseous products of denitrification by a bacterial culture (Allen and Van Niel, 1952; Sacks and Barker, 1952).

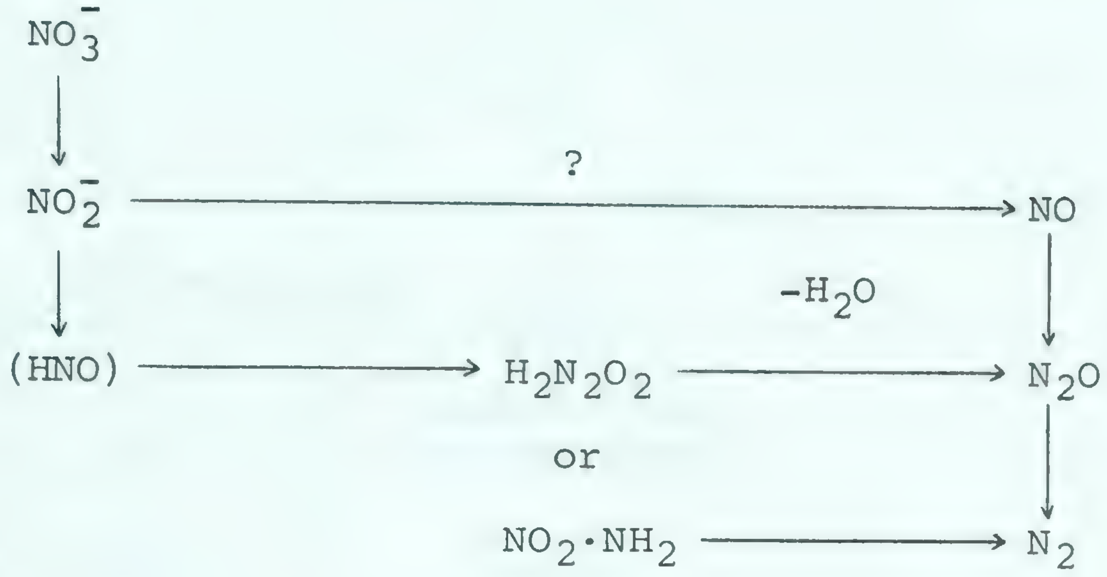
Valera and Alexander (1961) carried on studies on the nutrition and physiology of denitrifying bacteria. Their results and observations are based on a collection of eighteen organisms obtained from several sources. They found positive correlation between the numbers of denitrifiers and pH. Most of the bacteria did not show much activity at low pH values, but they showed optimum activity between pH 6.0 and 8.0. The effect of acidity on denitrification may be exerted in a number of distinctly different ways, and the sparse denitrifying population in acid environment may be a reflection of an influence

upon growth of responsible bacteria rather than an effect upon the denitrification mechanism itself. On the basis of extensive nutritional tests, these workers divided the N_2-producing microflora into four nutritional groups of varying complexity. Because of their nutritional and physiological differences, it is not surprising that a single medium is inadequate for the rapid growth of all.

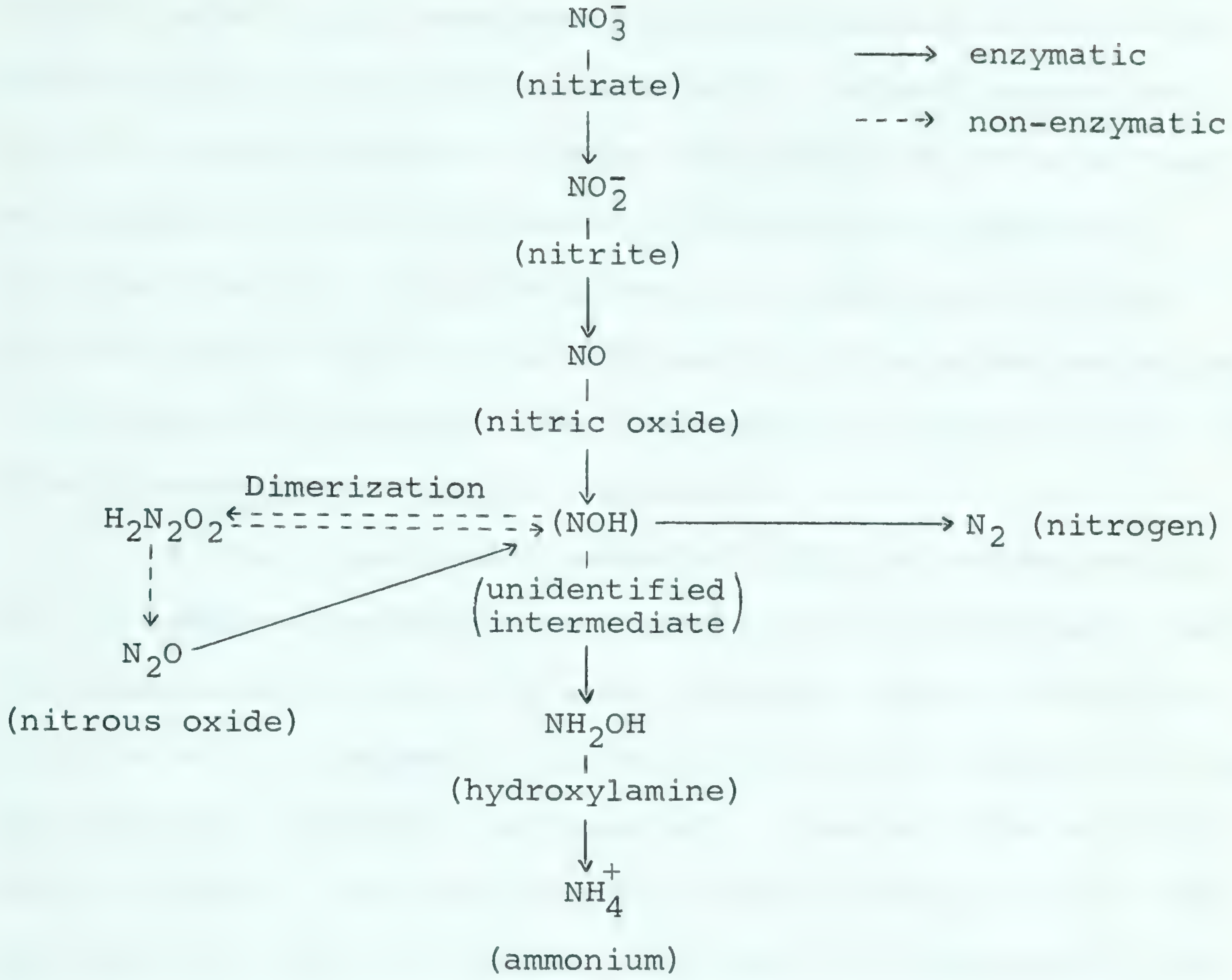
Biochemistry of denitrification has been extensively studied, and on the basis of accumulated data on different organisms, workers in this field have formulated theories and proposed schemes showing the step-wise reactions. Kluyver and Donker (1926) were among the first to propose a comprehensive scheme of reduction of nitrate to elemental nitrogen (i.e. denitrification), which was generally accepted until recent times. Their scheme is as follows:



Nason and Takahashi (1958) concluded that the biochemical pathway for denitrification is essentially as follows:



Fewson and Nicholas (1961) have presented an up-to-date scheme of general nitrate reduction in microorganisms. Their scheme is based on most recent knowledge about the intermediates in these processes. Nitric oxide is now considered to be a definite intermediate. An unidentified intermediate with empirical formula $N_2H_2O_2$ was thought by Kluyver and Donker to be hyponitrite. However, it has been proven by several workers (Allen and Van Niel, 1952; and Sacks and Barker, 1952) that hyponitrite and its stable isomers, nitramide and imidonitrite are not intermediates so the exact chemical structure of the compound $N_2H_2O_2$ remains uncertain. Their scheme is as follows:



Laboratory Studies on the Loss of Nitrogen From Soil

(a) Techniques

Unlike other nutrients, loss of nitrogen from soils cannot be easily estimated. One of the main reasons is that nitrogen is primarily lost as nitrogen gas, and the air itself is four-fifths nitrogen. Moreover, mineral nitrogen is transformed easily into other compounds or assimilated by microorganisms and higher plants causing immobilization. In some cases, free-living fixers may be simultaneously enriching the soil with nitrogen. In view of these circumstances, indirect

methods for measuring total loss have had to be used. Earlier workers based their measurement of nitrogen loss from soil on the analysis of nitrogen by the Kjeldahl method, before and after a certain interval of time, and designated this as loss of nitrogen by denitrification. If leaching is prevented, nitrogen loss due to denitrification on addition of nitrate (or nitrite) to soil can be followed by nitrogen determinations if the method of analysis used is capable of recovering all the likely non-gaseous nitrogen compounds.

In the conventional method of nitrogen determination, only nitrogen in ammonium and organic forms is included. Because the proportion of other inorganic nitrogen forms like nitrate, nitrite, etc. in normal field soils is low, the results for many purposes are reasonably satisfactory. However, when studying denitrification, determinations of total nitrogen in soil must include all forms of nitrogen. Although this approach is incapable of accounting for any increment due to accompanying nitrogen fixation, it is considered unlikely that significant fixation of nitrogen will occur during the process of denitrification since it is well known that nitrogen fixation is suppressed by small concentrations of nitrate. Bremner and Shaw (1958) have reported that in 1929, Olsen had described a method for the determination of total nitrogen in soil involving oxidation of nitrite to nitrate by acidified permanganate followed by reduction of nitrate to ammonia with reducing iron and sulphuric acid and subsequent digestion with concentrated sulphuric acid and catalyst and the normal Kjeldahl procedure. This method was slightly modified by them and found

to give very satisfactory results.

Loss of nitrogen through denitrification may also be measured by determining the amount of nitrogenous gases evolved over a certain interval of time. Some of the evolved gases like NO and NO_2 can be trapped in suitable absorbents and chemically determined. Such methods have been used in the past by some workers (e.g. Wagner and Smith, 1958). The analysis of evolved gases can also be carried out by gas chromatography or by mass spectrometer and infra-red techniques. In the laboratory, closed systems with arrangements for collection of any gas evolved were tried by early workers (Russell and Richard, 1917). A similar arrangement was designed by Wijler and Delwiche (1954) with added advantage of the use of the heavy isotope of nitrogen, N^{15}.

Gilmour, Damsky and Bollen (1958) and several other workers have used the Warburg apparatus for measuring the amount of nitrogen gas evolved during the process of denitrification. McGarity, Gilmour and Bollen (1958) used an electrolytic respirometer to allow control of partial oxygen pressure in their study of denitrification in soil. All the above methods enable the measurement of gas evolution directly from soil.

The study of denitrification in the field is beset with experimental difficulties that have so far proved insuperable and therefore most of the information now available has been obtained from laboratory experiments. One method of studying soils with a growing crop is that of pot-cultures in the greenhouse, although it must be accepted that there are numerous difficulties with this method. Such factors as the size of the

container, the nutrient supply, which may be too low or improperly applied, the soil moisture condition and poor soil structure leading to inadequate aeration and improper bacterial activity (Cook and Millar, 1946). A more accurate idea of what is happening to nitrogen in soils can be obtained by studies of individual experiments where soils have been maintained under near-natural conditions, and nitrogen income and outgo measured over a long period of years. Such data can be obtained only from lysimeters, where the drainage water can be collected and analyzed.

Use of labelled nitrogen in any of the methods mentioned so far, provides greater accuracy and precision. Without labelled nitrogen, it is necessary to add nitrogen to the soil in amounts considerably greater than those applied in the field. Use of labelled nitrogen can minimize the error due to nitrogen fixation and such addition will not be accounted for in the labelled products of denitrification. It also affords the opportunity to clearly know about the interconversion of added nitrogen to different forms of nitrogen in the soil. According to Broadbent and Stojanovic (1952), the use of a tracer in studying nitrogen transformation in soils is particularly advantageous, since all soil nitrogen is organic, and major changes are not obscured by complicating side reactions with the inorganic soil constituents. Dilz and Woldendorp (1960) used N^{15} in grass sods in a short-time experiment and could draw a balance-sheet of the added fertilizer, after accounting for the fertilizer nitrogen in tops, roots and soil. They found that in the case of nitrate application on grassland, 5-30 per

cent of the nitrogen in the soil may be lost depending on soil and climatic factors.

(b) Factors Affecting Denitrification

Laboratory studies on the process of denitrification in different soils under controlled conditions have lead to the following conclusions with regards to the factors affecting this process:-

pH

The rate of denitrification is profoundly affected by the pH of soils. In the soils which Bremner and Shaw (1958) were studying, they found that denitrification was slowest in soils of pH 3.6 to 4.8, quite rapid with soils having pH 8.0 to 8.6, and almost all the added nitrate was lost within 4-8 days in the case of Weald silty clay having a pH of 7.5. Wijler and Delwiche (1954) reported that total denitrification rate was quite constant above pH 6, but the proportion of N_2O and N_2 were pH dependent. Above the neutral point, nitrous oxide could be readily reduced to nitrogen. Below pH 7, the reduction of N_2O was strongly inhibited. Below pH 6, the formation of NO became pronounced although the rate of denitrification was low at pH 5 and below. By raising the pH, the rate of denitrification was increased and the optimum was found to lie between pH 7-8. A further increase in pH suppressed denitrification and almost ceased at pH values above 10.5. Nommik (1956) also found that under completely anaerobic conditions ($pO_2 < 2$ mm. Hg), there was a similar connection between pH and rate of denitrification, although the influence of pH

was less pronounced. Several other soils showed a similar trend. Carter and Allison (1961) found that losses of nitrogen by volatilization from soils having pH value between 5.4 and 5.8 were negligible during incubation as well as during air-drying following incubation. When these soils were limed to pH 6.7, a loss as high as 15 per cent of added nitrogen was found.

Temperature

Denitrification has been found to increase rapidly with rise in temperature from 2° to $25^{\circ}C$. The optimum temperature was $60^{\circ}C$ and the maximum $70^{\circ}C$ (Bremner and Shaw, 1958). According to Alexander (1961), the effect of low temperature on the rate of denitrification is of agronomic importance because it indicates the possibilities of nitrogen volatilization during the winter months when crops are not utilizing the nitrate formed by nitrification. Since denitrification has been found to be rapid at elevated temperatures, the thermophilic flora must be active in carrying on denitrification. This aspect of the problem requires investigation.

Moisture Content

Moisture content of soil has a direct effect on the rate of denitrification, and this aspect has attracted considerable attention by many research workers. It is most likely that denitrification occurs in soils with a high moisture content because, in such a situation, the oxygen supply is markedly restricted.

Bremner and Shaw (1958) confirmed that the rate of

denitrification of nitrate in the soil is profoundly affected by the water content of the soil. Even under most favorable conditions, little loss of nitrogen occurs if the moisture content is less than 60 per cent of the water-holding-capacity of the soil. Nommik (1956) also observed the same trend. At lower moisture content, the denitrification gas consisted chiefly of N_2O, suggesting that degree of anaerobic condition is one of the factors which determines the composition of the products of denitrification. Wijler and Delwiche (1954) claimed that the moisture content of soil affected denitrification by inhibiting the diffusion of oxygen into the soil.

McGarity (1961) observed that the gas-evolution curve indicated an initial lag period followed by a steady rate of gas evolution until the added nitrate is depleted in all the treatments except that having the lowest moisture content (near wilting point with 8.7 per cent moisture). McGarity's findings were on the basis of gas evolved in Warburg flasks incubated under completely anaerobic atmosphere (argon gas instead of air). However, his results indicate an increased rate of denitrification with increments of soil water content. Cady and Bartholomew (1960) also reported the pronounced effect of moisture content on denitrification in soils incubated in an atmosphere of argon.

Oxygen Tension

Soils saturated with water have been found to show greater denitrification loss of nitrogen than well-aerated soils. This observation can be explained on the basis of lack of free oxygen in water saturated soil, so much so that the

soil microorganisms cannot meet their oxygen requirements and the denitrifiers utilize nitrate instead of oxygen and thus cause denitrification. Nommik (1956) found that in the absence of oxygen, in argon atmosphere, the rate of denitrification was extremely high, but when the partial pressure of oxygen was as high as 760 mm. Hg, denitrification failed to take place altogether. Nommik also studied denitrification with different sizes of soil aggregates, because they influence the exchange of gases in soils. He found that in soils consisting of coarse aggregates, denitrification is at a considerably lower level, suggesting free gas movement caused oxygen availability to the soil microorganisms.

Bremner and Shaw (1958) incubated water-logged soils containing nitrate and glucose in 300 ml. Kjeldahl flasks stoppered by rubber bungs carrying two glass tubes fitted with taps which permitted evacuation and replacement of air in the flasks by oxygen or nitrogen. Flasks were filled with air, oxygen and nitrogen. Four flasks having each type of atmosphere were not shaken and the other four were shaken for eight hours each day. They found that if the flasks were not disturbed during incubation, the loss was practically the same in the evacuated flasks as in those containing nitrogen or air and was much greater in these than in flasks containing oxygen. Shaking greatly reduced denitrification in the flasks containing air and completely inhibited denitrification in the flasks containing oxygen.

The results of these workers and others (Carter and Allison, 1960) indicate that under aerobic conditions, de-

nitrification is not possible. This view is, however, not fully shared by some other workers in the field. Broadbent (1951) observed nitrogen losses at all oxygen levels when both nitrate and ground clover were added to samples of sandy soil. Broadbent and Stojanovic (1952) found that although denitrification of added nitrate was inversely related to partial pressure of oxygen, it was of appreciable magnitude even under fully aerobic conditions. Wijler and Delwiche (1954) also found that oxygen has a pronounced suppressing effect on the process of denitrification and oxygen tension as low as 5 mm. Hg decreased denitrification rates to almost one-tenth of the anaerobic denitrification. McGarity et al. (1958) observed small losses under fully aerobic conditions and large losses under anaerobic conditions. Allison et al. (1960) observed a trace of nitrate reduction when Branchville sandy loam was incubated at approximately one-third atmosphere moisture content, even in the presence of one per cent glucose or 2 per cent wheat straw. When the soils were aerated for one to three weeks with nitrogen gas containing 27 per cent oxygen, the loss of nitrogen was significant in about half the tests and not in others. When the oxygen partial pressure was only 0.46 per cent, the losses of nitrogen were as high as 10 per cent of the added nitrate even in the absence of added energy source and about 50 per cent of the added nitrate in the presence of 0.5 per cent glucose. In a recent study, using widely differing soils, Greenwood (1961) found that the change-over from aerobic to anaerobic metabolism of organic materials takes place at an oxygen concentration less than 3×10^{-6}M. McLaren

(1963) has therefore remarked that water saturated crumbs of more than about 3 mm. in radius have no oxygen at their centers. Since crumbs of this size are present in many soils, it means that pockets without oxygen are probably ubiquitous in soils. These spots are thus likely to be the usual seat of anaerobic processes in soils including denitrification. It is thus possible that nitrification of ammonia to form nitrate and denitrification of nitrate resulting in nitrogen can go on simultaneously in the soil. The denitrification under aerobic conditions claimed by various workers can be explained in this fashion. However, it must be assumed that nitrate or nitrite produced in aerobic spots must move to anaerobic spots in order to be reduced. This has not been demonstrated conclusively to date. Another alternative suggestion is that the same spots alternatively become aerobic and anaerobic. This line of reasoning has been proposed by most of the contemporary workers in this field.

Cady and Bartholomew (1961) believed that volatile losses of nitrogen oxides and/or nitrogen gas do take place in the presence of oxygen under certain environmental conditions. They suggested that the term "aerobic denitrification" has to be considered in relation to the level of biological pressure. They incubated soil samples in an enclosed apparatus maintained at 754 ± 0.5 mm. Hg pressure and $28^{\circ} \pm 1^{\circ}C$ temperature with arrangements for continuous constant pressure of oxygen. Gaseous phase was periodically examined for oxygen and nitrogen products, and on the basis of the results obtained, they concluded that with an available energy supply, a microbial build-up resulted

which presumably included numerous denitrifiers. Under these conditions and at low levels (0.1 to 1.6 per cent) of free oxygen in the atmosphere of the apparatus, combined oxygen in the form of nitrate was used as a hydrogen acceptor at the same time free oxygen was used. When the biological pressure was reduced following the extensive decomposition of the added plant residue, the total need for oxygen was decreased and the free oxygen supplied an adequate amount of hydrogen acceptor resulting in its use in preference to the combined oxygen.

Partial pressure of oxygen has been found to have considerable influence on the end gaseous product of the denitrification process. Wijler and Delwiche (1954) reported three gaseous products, namely N_2O, NO and N_2, identifiable in a suitable denitrifying soil environment. Nitrous oxide under suitable conditions can be further reduced to nitrogen. They were, however, of the opinion that under most soil conditions, N_2O may be the major product, and they observed this gas being produced from soils containing as little as 3.4 μe. of NO_3^--N per g. of dry soil. The question that nitrous oxide is the obligatory precursor of molecular nitrogen in the process of denitrification has been examined by Nommik (1956) and his results inclined him to accept that it is so. Cady and Bartholomew (1960) examined the sequential products of aerobic denitrification in soil from the Ap horizon of a Norfolk sandy loam and reported NO as the initial product of denitrification. This never exceeded 5 per cent of the tagged nitrate added to the soil. They found that the presence of nitric oxide was closely associated with the presence of nitrite in the soil.

They concluded that the NO detected must have evolved from the decomposition of nitrous acid. Subsequent to the appearance of NO, nitrous oxide appeared in the gaseous phase which coincided with a decrease and eventual disappearance of NO. Following the increase in N_2O, was the appearance of nitrogen gas and this gas went on increasing until nitrous oxide disappeared from the gas phase. The same workers, in a subsequent paper (1961), reported that in the presence of oxygen, NO was not produced because NO in such a case combines with oxygen to form NO_2 and other products and recycles to nitrate, unless the soil system is dry enough to permit gaseous diffusion and escape of NO_2.

Organic Matter

Most of the denitrifying organisms are heterotrophs, although examples of chemoautotrophs capable of reducing nitrate to molecular nitrogen are not lacking. It is therefore understandable why denitrification cannot occur in soils without the substrate containing some organic compounds which support growth of the organisms and act as hydrogen donor. Nommik (1956) mentions that in 1895, Wagner drew attention to the great risk of denitrification if the soil was simultaneously supplied with large quantities of nitrate and stable manure. Thus a readily available supply of carbonaceous material is essential for denitrification. Jansson and Clark (1952) working with plant material found that alfalfa when decomposed at two -thirds moisture saturation lost considerable nitrogen, but not the wheat straw. However, addition of sugar or peptone, increasing the water content to saturation, and more finely-ground

plant material, made it possible to observe biological denitrification even in wheat straw. Wijler and Delwiche (1954) found that although the use of straw instead of alfalfa as a substrate reduced the rate of denitrification, the proportion of N_2 and N_2O formed were essentially unchanged.

Bremner and Shaw (1958) added different amounts of glucose to water-logged soil containing fixed levels of added nitrate and found that on incubation maximum loss of nitrogen occurred when the ratio of carbon added as glucose to nitrogen added as nitrate was 2:1 to 3:1. Only one of their soils did not conform to this trend and this soil had a lower organic carbon content. They also found that readily decomposable organic compounds like glucose induced rapid denitrification of nitrate. A comparison of different carbonaceous materials like cellulose, lignin, sawdust and grass, when added to water-logged soil containing nitrate indicated that the rate of denitrification varied with their resistance to decomposition by soil microorganisms; being most rapid with cellulose and least with lignin and sawdust.

McGarity (1961) also reported that the level of assimilable organic carbon is a major factor in determining the maximum level of nitrogen gas evolution. In his experiments with different levels of assimilable organic carbon, he observed that once a critical level is reached, any further increase did not cause any appreciable rise in the maximum rate of denitrification.

Concentration of Nitrate

Wijler and Delwiche (1954) are of the opinion that

initial nitrate concentration did not influence the rate of denitrification. Nommik (1956) also agreed with Wijler and Delwiche. According to Nommik, at high concentration of nitrate, the denitrification gas chiefly consisted of nitrous oxide and the reduction of nitrous oxide to molecular nitrogen does not seem to start until the greater part of the nitrate has been consumed. Cooper and Smith (1963) observed that decreasing the initial concentration of added KNO_3 did not change the overall rate of denitrification. They also found a marked reduction in maximal amounts of N_2O found in the gaseous atmosphere when the initial concentration of KNO_3 was reduced. McGarity (1961) found that over a very wide range of nitrate concentration, the initial concentration of nitrate does not influence the maximum rate of denitrification. Bremner and Shaw (1958), using different amounts of nitrate added to a fixed weight of soil, and also fixed amount of nitrate to different weights of soil, found that loss of nitrogen after 10 days of incubation, calculated as a percentage of NO_3^--N added, did not vary with the level of nitrate. In the case of too low levels of nitrate, determinations of total N could not accurately measure the loss involved because of the small differences involved in the two treatments. They also reported that the rates of denitrification of potassium, sodium, strontium and calcium nitrates were practically the same, but the loss of nitrogen was more rapid with ammonium nitrate and exceeded 100 per cent when calculated as a percentage of the added NO_3^--N, which shows that some ammonium was nitrified and the nitrate and/or nitrite formed was itself denitrified.

Greenhouse and Field Studies

As already mentioned, accurate soil nitrogen losses cannot be measured from the experimental data obtained in ordinary field conditions. This is chiefly due to lack of knowledge of the quantity of nitrogen removed annually by leaching and erosion, frequent failure to analyze the crops for total nitrogen, and the uncertainty as to the contribution that the sub-soil has made to the feeding of the crop. Nevertheless, field experiments do supply information that is sufficiently quantitative to show the magnitude of the main losses or gains. The experiments conducted on the Broadbalk wheat field at Rothamsted, as reported by Russell (1961), for the unmanured plot kept under continuous wheat for 49 years (1865-1914) gives an example of the full nitrogen loss from the soil being unaccounted for by the nitrogen in the crop removed. When nitrogen was added either as sulphate of ammonia, or as farmyard manure, very large losses occurred, no less than 70 per cent of the nitrogen in the farmyard manure being unaccounted for. Some of this loss undoubtedly occurred in the drainage water, which could not be estimated accurately in the field experiment, but it was considered very unlikely it could account for any appreciable proportion of it.

These unaccounted for losses of nitrogen also occur when grassland is converted to arable, or when virgin prairie soil is broken up for cropping. Shutt (1910) reported large losses in the early twenty two years of breaking up the prairie at Indian Head, Saskatchewan. In the next sixteen years, Hopkins and Leahey (1944) could account for almost the

whole of the loss of soil nitrogen by the wheat crop taken. Caldwell, Wyatt and Newton (1939) examined the loss of nitrogen in addition to other nutrients from soils being used for grain production in Canadian prairie soils and found that the surface six inches of cultivated black, dark brown, brown and grey soils have lost on the average 1,775, 1,145, 765 and 723 pounds of nitrogen per acre respectively, which is equivalent to a decrease of 18 per cent, 22 per cent, 20 per cent, and 30 per cent of the original nitrogen content as measured in the virgin sod. These workers attributed this loss to soil drifting, because, according to them, leaching processes are not believed to take place in western Canada in view of low precipitation and high evaporation in this region, and they were not sure, in view of lack of experimental evidence, that nitrogen loss by volatilization could take place in these soils.

Lysimeter studies have also shown large unaccounted for nitrogen losses. Allison (1955) has very exhaustively discussed quite a number of results obtained by lysimeter studies in the United States and concluded that a large proportion of the nitrogen not recovered in the crop was found in the leachate, but substantial unaccounted for losses occurred in most lysimeters.

Greenhouse studies as a supplement to field studies can be of substantial value in evaluating the nitrogen losses by volatilization. Allison (1955) has described the experiment of Pinck, Allison, and Gaddy (1948), which extended over a period of five years, and on the basis of data obtained, it was found that the unaccounted for nitrogen amounted to 18 per

cent. For higher rates of application, the overall loss was near 14 per cent. Allison feels that an ideal experiment for checking denitrification or volatile losses of nitrogen in greenhouse pots, is to have several applications of nitrogen to the same soil, several non-legume crops grown, and all soils and crops analyzed. He felt that very few experiments reported in literature meet these requirements.

EXPERIMENTAL

Materials

(a) For Greenhouse Experiments

Surface soil samples (0-6 in. depth) from seven sites in different soil zones of Alberta were collected for the first set and from two sites for the second set of experiments. The locations of these sites are shown in Fig. 1 and some information about these soils is given in Appendix A. For Set No. 1, about 45 kg. of soil from each site was collected in July-August 1963, and for Set No. 2, 55 kg. of soil from the two sites was collected in May 1964. These soils were air dried and then passed through a 10-mesh sieve before use.

(b) For Denitrification Studies in Warburg Apparatus

For this aspect of study, some 50 soil samples from different sources were collected. Composite surface samples from plots in the field were obtained by using a soil auger. The collection of samples from profiles was carried out with the help of a Bull core sampling machine*. When a whole core was taken out, the surface of the core was scraped with a sharp sterile knife and on the newly exposed surface, a sterile cork borer was used to collect samples from different horizons. Sampling was always done from lower horizons upward, and after

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* Product of A.D. Bull Enterprises, Chickasha, Oklahoma, U.S.A.

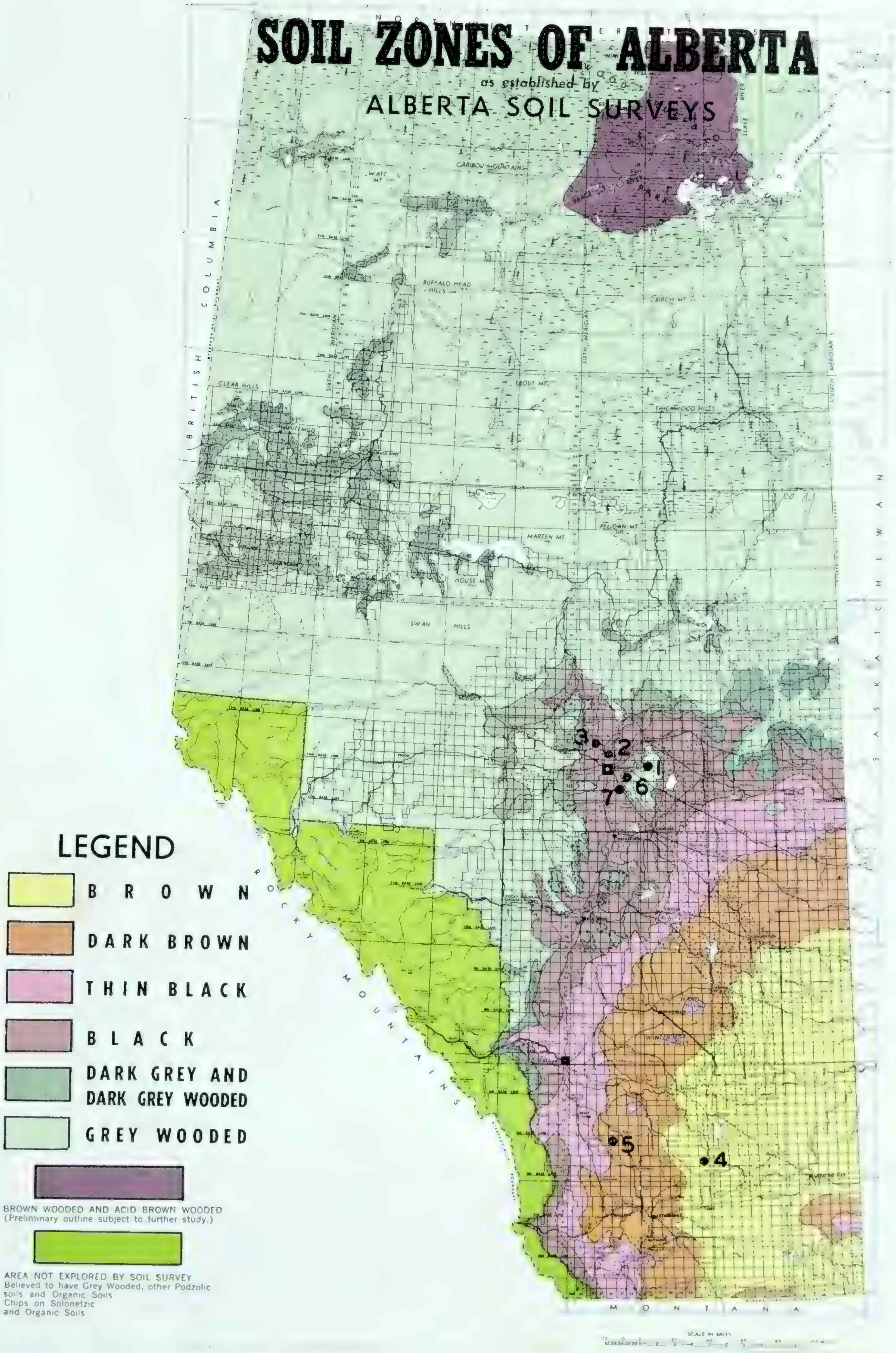

Figure 1. Locations of the Sites of Soil Sampling.

(Other Details of the Sites are in Appendix A)

getting the sample from a horizon and putting it in a sterile, marked glass bottle, the sampling equipment was thoroughly cleaned and washed with alcohol for sterilization.

The following samples were used:

1. Seven surface samples from cultivated fields in the different soil zones of Alberta (see Fig. 1 and Appendix A.).
2. Soil samples from the horizons of seven profiles on virgin lands obtained in April 1964 from the sites described in Appendix A. The details of horizons are described in Table X.
3. Soil samples from the plots of A-B-C Dryland Project, Lethbridge Research Farm for 1953 and 1963.
4. Soil samples from some Eight years Rotation Plots, Lethbridge Research Farm, for 1955 and 1963.
5. Soil samples from some plots of Project 10.01.10 (old project 6.1.6) of Lacombe Research Farm for 1955 and 1963.
6. Soil samples from Rotation Plots, Beaverlodge, collected in 1961.

Methods

(a) Greenhouse Experiments

Two sets of greenhouse experiments were conducted for the study of nitrogen loss. The details of the two sets are as follows:

	SET NO. 1	SET NO. 2
SOILS	From Sites 1-7	From Sites 1 and 3
TREATMENTS	Six nitrogen carriers, namely Ammon. sulphate, Ammon. nitrate, Sodium nitrate, Ammon. phosphate, Urea, and Dried blood at the rate of 40 mg. N/100 g. without plants.	Three nitrogen carriers, namely Ammon. sulphate, Sodium nitrate, and Urea at the rate of 40 mg. N/100 g. with and without barley plants.
REPLICATIONS	Four	Five
DESIGN	Completely randomised. Pots were rearranged in greenhouse, at random, every week.	Completely randomised. Pots were rearranged in greenhouse according to a table of Random Permutations every week.
DATE STARTED	October 10, 1963	May 29, 1964
DATE TERMINATED	March 7, 1964	July 17, 1964

Exactly 1.5 kg. of soil and requisite amounts of nitrogen carrier equivalent to 40 mg. N/100 g. of soil in dry condition were mixed in a "batch mixer". The soil was then put in a properly marked pot. The pots used in both sets had

their drainage holes plugged to eliminate any drainage loss of nitrogen. Distilled water was added to the pots by weighing so as to bring them to 1/3rd atmos. moisture tension; water was not added to check pots. In Set No. 2, ten barley seeds were sown about 1 inch deep in the appropriate pots before watering. Subsequently, water was added in all the pots, except check pots, at regular intervals of 4 to 6 days to bring the pots up to weight.

For sampling, the whole bulk of soil from the pots was dumped on thick waxed paper, allowed to air dry, crushed and passed through a 2 mm. sieve. Pots with barley plants were treated the same way after the plants with roots were gently removed by shaking and washing in a minimum quantity of water to cause as little destruction of the root system as possible, and the shaken and washed-off soil returned to the soil from the pot. Sub-sampling from each pot was done by using a sample-splitter to get about 100 g. of soil from each pot, and transferred to labelled glass bottles with lids.

In Set No. 2, where some of the pots had barley plants, the plant materials were dried at 70° C, weighed and then ground in a Wiley cutting mill 475A before storage.

(b) Determination of Total Nitrogen in Soils

Olsen's method as modified by Bremner and Shaw (1958) was used for the determination of total nitrogen, including NO_3^--N and NO_2^--N, in soils. Since this method is the basis for evaluating the nitrogen loss from soils in this study, it is described below:

Three to five g. of soil, weighed to the nearest mg.,

were transferred to a Kjeldahl flask (800 ml.) and treated with 10 ml. of 5 per cent (w/v) $KMnO_4$ and then 20 ml. of 50 per cent (v/v) H_2SO_4 was added slowly with shaking. After about 5 minutes, two drops of octanol and 5 g. of reduced iron powder were added. After the initial effervescence subsided, the flask was shaken and then heated gently for about 45 minutes, care being taken to ensure that only slight loss of water by evaporation took place. The flask was then allowed to cool and then 10 g. K_2SO_4, 1 g. $CuSO_4 \cdot 5H_2O$, and 0.1 g. selenium powder and 60 ml. conc. H_2SO_4 were added. The mixture was then heated until it assumed a yellowish-green color and was subsequently boiled for 5 hours. The flask was allowed to cool, 200 ml. of distilled water added and again cooled, 200 ml. of 40 per cent NaOH added, and then distilled with the evolved ammonia being collected in 25 ml. of 4 per cent boric acid solution. This solution was then titrated, using mixed indicator composed of methyl red and bromcresol green, with standard acid.

(c) Determination of Denitrifying Parameters of Soils, Using Warburg Apparatus

The terms "Denitrifying potential" and "Denitrifying capacity" will be used as parameters for comparing different soils. These parameters were determined as follows:

A 5.0 g. sample of soil was placed in a 25 ml. Warburg flask. Then 2.0 ml. of KNO_3-glucose solution, containing 2 mg. of NO_3^--N and 50 mg. of carbon as glucose, were applied dropwise to the soil, using a hypodermic syringe. Where necessary, an

additional known quantity of distilled water was added to bring the soil to approximately field capacity. Three-tenths ml. of 10 per cent KOH solution and a piece of folded filter paper were placed in the center well. After the flask was attached to the manometer, the system was evacuated and filled with argon, bubbled through chromous chloride. This gassing procedure was repeated five times. The manometers were placed in a constant temperature bath (30° C) and readings taken every 3 to 6 hours and recorded, as in the normal Warburg manometric method (Umbreit, Burris and Stauffer, 1959) for evolution of gas. A sample of the data sheet and calculation is presented as Appendix B. Since gas is evolved and the fluid rises in the open arm of the manometer, gas from the flask was periodically released, taking precautions that air did not get in, to enable the readings to be continued until there ceased to be any further evolution of gas in the flask. The volume of gas evolved and the time taken were used to compute the per cent of NO_3^--N lost (capacity) and the rate of nitrogen evolution (potential) for the sample, as follows:

Denitrifying Capacity (D.C.) = Nitrogen evolved as gas, expressed as percentage of added NO_3^--N.

Denitrifying Potential (D.P.) = μg. of N_2 evolved/g. of soil/hour.

(d) Estimation of Counts of Denitrifying Bacteria in Soils

Numbers of denitrifying bacteria were estimated by the most probable number (MPN) method, using the production of gas

and an alkaline reaction as an indication of denitrification (Prescot, Winslow and McCrady, 1946; Timonin, 1946; and Valera and Alexander, 1961). The method was as follows:

Giltay's medium (Waksman, 1927), with the following composition, was prepared: Solution I, distilled water 250 ml., KNO_3 2 g., asparagine 1 g.; Solution II, distilled water 500 ml., citric acid 5 g., KH_2PO_4 2 g., $MgSO_4$ 2 g., $CaCl_2$ 0.2 g., $FeCl_3$ trace. Solution II was neutralized with a 10 per cent KOH solution having 5-6 ml. of 1 per cent bromthymol blue as indicator. The two solutions were then mixed and made to one liter with distilled water. One ml. of ten-fold dilutions of a soil sample was used for inoculating five test tubes containing 10 ml. of Giltay's medium. Further series of dilutions up to 10^{-7} or 10^{-10}, depending upon type of soil, were made and each dilution was used to inoculate five test tubes with 10 ml. of medium. The cultures were incubated at 30° C and observed daily for gas production. During denitrification, the medium becomes alkaline and the color changes from green to intense blue (above pH 7.6). Final reading was found to take 48 to 60 hours, depending on soil and number of organisms present. The last dilution having all positive tubes and the succeeding three dilutions were selected as the significant dilutions for the estimation of MPN, which was read from the table prepared by Hoskins (1934, as reproduced by Prescot, Winslow and McCrady, 1946). The validity of MPN (most probable number) has been tested by numerous workers, including statisticians, water bacteriologists and microbiologists, and found to be statistically sound.

(e) Other Determinations

pH

Ten g. of soil sample and 10 ml. of distilled water were mixed with a stirrer and the acidity of the mixture was measured on a Beckman Model H-2 pH meter, equipped with glass and calomel electrodes.

Organic Nitrogen in Plant Material

Total nitrogen was determined by the A.O.A.C. (1955) macro-Kjeldahl procedure using 10 g. of catalysts K_2SO_4-$CuSO_4$ -Se mixture in proportion of 100:10:1 by weight. Distilled ammonia was collected in a four per cent boric acid solution as suggested by Meeker and Wagner (1933) and titrated with standard H_2SO_4 using a mixed indicator (methyl red and bromcresol green).

Nitrate Nitrogen

Nitrate nitrogen was determined by phenoldisulphonic acid method (Jackson, 1958). The actual amount of nitrate was determined from a standard curve prepared by taking different aliquots of standard KNO_3 solution (0.7214 g./liter) and making up to volume in 100 ml. volumetric flasks. The standard curve thus prepared is shown in Appendix C.

Field Capacity

This was determined in a pressure cooker when one-third atmosphere pressure, equivalent to 5 lb./in.^{2}, was applied and moisture in the soil determined and expressed as percentage of dry weight of soil.

Mechanical Analysis

Mechanical analysis of soil was determined by the hydrometer method (Bouyoucos, 1951).

PRELIMINARY TESTS ON METHODS

Some preliminary experiments were carried out with soils available in the laboratory and one collected from flower beds on the campus and designated as "Garden soil" to test the validity of the methods described in the previous section. Some characteristics of the soil used were determined and found to be as follows:

Soil	Texture	pH	Organic* Matter %	Total Nitrogen %	Field Capacity %
Malmo	CL	6.3	13.6	0.59	34.0
Podzol B	S	5.3	2.8	0.018	5.6
Garden soil	SiC	6.7	14.2	0.60	45.3

* Data for organic matter reported by the Soil Science Department.

(a) Recovery of Added Nitrogen

The object of this series of experiments was to determine if total nitrogen estimation, according to 'modified Olsen's Method', is accurate enough to measure the loss of nitrogen from added nitrogen sources in the greenhouse experiment. Nitrate, nitrite and ammonium forms of nitrogen were added at the rate of 100 and 20 mg. N/100 g. to 5 g. of soil and total nitrogen in the whole content determined immediately. The results, representing the average of 6 determinations, are presented in Table I.

In a second experiment, 5 g. samples of soil taken in

TABLE I

Recovery of Added Nitrate, Nitrite and Ammonium Nitrogen to Malmo and Podzol B Soils

Soil	Nitrogen in Soil (mg./100 g.)	Nitrogen Added (mg./100 g.)	Total Nitrogen (mg./100 g.)			Recovery of Added Nitrogen %
			Calculated	Range	Found	
Malmo	583	100 as nitrate	683	(679-687)	684	101
	583	100 as nitrite	683	(682-687)	685	102
	583	100 as ammonium	683	(681-685)	682	99
Malmo	588	20 as nitrate	608	(606-610)	608	100
	588	20 as nitrite	608	(608-612)	609	105
	588	20 as ammonium	608	(608-610)	609	105
Podzol B	19	20 as nitrate	39	(37-40)	38	95
	19	20 as nitrite	39	(36-40)	39	100
	19	20 as ammonium	39	(34-42)	39	100

Kjeldahl flasks were treated with 2,000 ppm. of nitrate-nitrogen as KNO_3 and sufficient glucose to result in a C/N ratio of added material equal to 2.5 and incubated at 30° C and under water-logged conditions (125 per cent of W.H.C.). Three flasks were taken out of the incubation chamber in the case of Malmo soil after 0, 1, 2, 4, 7, 14, 21 and 30 days, and Podzol B after 0 and 14 days, and total nitrogen determination was carried out. This experiment was performed three times and only close values obtained are reported in Table II.

It is evident from Tables I and II that the method is accurate enough to measure losses of nitrogen. The above results were obtained on moist soils so there seems to be no need to follow the usual practice of drying samples before determination of total nitrogen. However, in the greenhouse experiments, sub-sampling was done from each pot after drying to obtain uniform samples.

(b) Use of Warburg Apparatus for Determining Denitrification Loss of Added Nitrate in the Presence of Oxidizable Carbon and Under Anaerobic Conditions

Nitrate-nitrogen at the rate of 40 mg. N/100 g. soil (equivalent to 2 mg. NO_3^--N for 5 g. of soil) and glucose at 125 mg. (equivalent to 50 mg. of C) were added to 5 g. of soil in the Warburg flasks. Glucose was provided so that energy source may not become a limiting factor, especially in soils low in organic matter. The possibility of nitrogen fixation was realized but was considered remote under the conditions of these experiments. Malmo and garden soils were used with and without nitrate in the initial experiments. The results obtained

TABLE II

Reproducibility of the Results of Incubation Experiments of Soil With Nitrate and Glucose Under Water-logged Conditions

Soil	Period of Incubation (days)	Total Nitrogen Content as mg.N/100 g. Soil		N Loss as % of Added NO_3^--N
		No. of Replications Reported	Average Value	
Malmo Soil	0	6/9	878	0
(pH 6.3)	1	6/9	817	30.5
	2	6/9	777	50.5
	4	6/9	765	56.5
	7	6/9	755	61.5
	14	6/9	713	82.5
	21	5/8	717	80.5
	30	6/9	708	85.5
Podzol Ae	0	6/9	211	0
(pH 5.3)	14	6/9	209	1.0
Podzol Ae	0	6/9	216	0
($CaCO_3$ treated; pH 7.4)	14	6/9	201	7.5

are presented in Table III.

It was observed that in all cases, there was an initial period of absorption of gas before the evolution of gas. When the corresponding volumes 'without nitrate' (i.e. check) are deducted from 'with nitrate' (i.e. treated), this initial absorption of gas is eliminated. So far as the reasons for such a phenomenon are concerned, nothing can be said definitely. It is possible there may be some entrapped air in the pore spaces of the soil even after complete evacuation (1.5 mm. Hg. pressure) and part of this is first used by the denitrifiers before utilizing NO_3^-. Another possible reason may be the time lag in the physical or surface absorption of the argon gas in the spaces from which air was evacuated. It was observed that a small amount of gas was evolved from soil without added nitrate in all cases, which is likely due to the small amount of residual nitrate present in most soils. Therefore, to compensate for the initial contraction in the gas volume of the flask and also for any increase in gas volume due to soil nitrate, it was considered appropriate to take the difference of the 'treated' and 'check' as the effective volume of gas evolved. Henceforth, denitrification parameters will always be presented after deducting the corresponding 'check' values.

The time taken to denitrify the added nitrate when no more evolution of gas could be observed, was also found to be different for different soils. Thus no time limit for any sample was possible. Incubation had to be continued until gas evolution ceased and the time vs volume of gas evolved curve flattened out.

TABLE III

Evolution of Gas in Warburg Flasks When 5 g. of Soil was Incubated With and Without 2 mg. NO_3^--N and 50 mg. C as Glucose in an Argon Atmosphere

(Values are Average of Three Determinations)

Soil	µl. of Gas (nitrogen) Evolved Without Added Nitrate	With Added Nitrate	Difference	Total Time (Hrs.)	Rate of Gas (nitrogen) Evolution (µl./g. of soil/hr.) Without NO_3^--N	With NO_3^--N
Malmo	165	1450	1285	85	1.50	6.75
Garden	80	1580	1500	66	0.80	9.86

An experiment with two levels of nitrate-glucose mixture added at different intervals was carried out next. A second dose of 2 mg. NO_3^--N was put in the side arms of the flask and was tipped into the soil when further evolution of nitrogen gas from the first dose of nitrate ceased. The data of this experiment are presented in Fig. 2.

The percentage recovery (D.C.) from the second instalment of nitrate-nitrogen is inconsistent, because in transferring nitrate-glucose solution from the side arm, a few drops of the solution adhered to the glass and did not reach the soil. Moreover, in transferring from the side arm, there was always the danger of spilling some KOH solution from the center well into the soil. It was, therefore, thought advisable to apply the nitrate-glucose solution uniformly at the start of the experiment and have a separate 'check' flask, rather than transfer the nitrate-glucose solution from the side arm.

Samples of gas were collected from the Warburg flasks which were treated with nitrate-glucose solution. These gases were analyzed in the mass spectrometer by Dr. P. Kebarle, Department of Chemistry, University of Alberta. The composition of the gas was found to be argon and nitrogen only, except in some samples where small amounts of CO_2 were found. The samples which showed these CO_2 peaks were from those flasks receiving two doses of nitrate and we were using 0.2 ml. of 5 per cent KOH in the central well of the Warburg flask. It was then decided to use 0.3 ml. of 10 per cent KOH and in subsequent samples, the CO_2 peaks disappeared. There was no trace of any

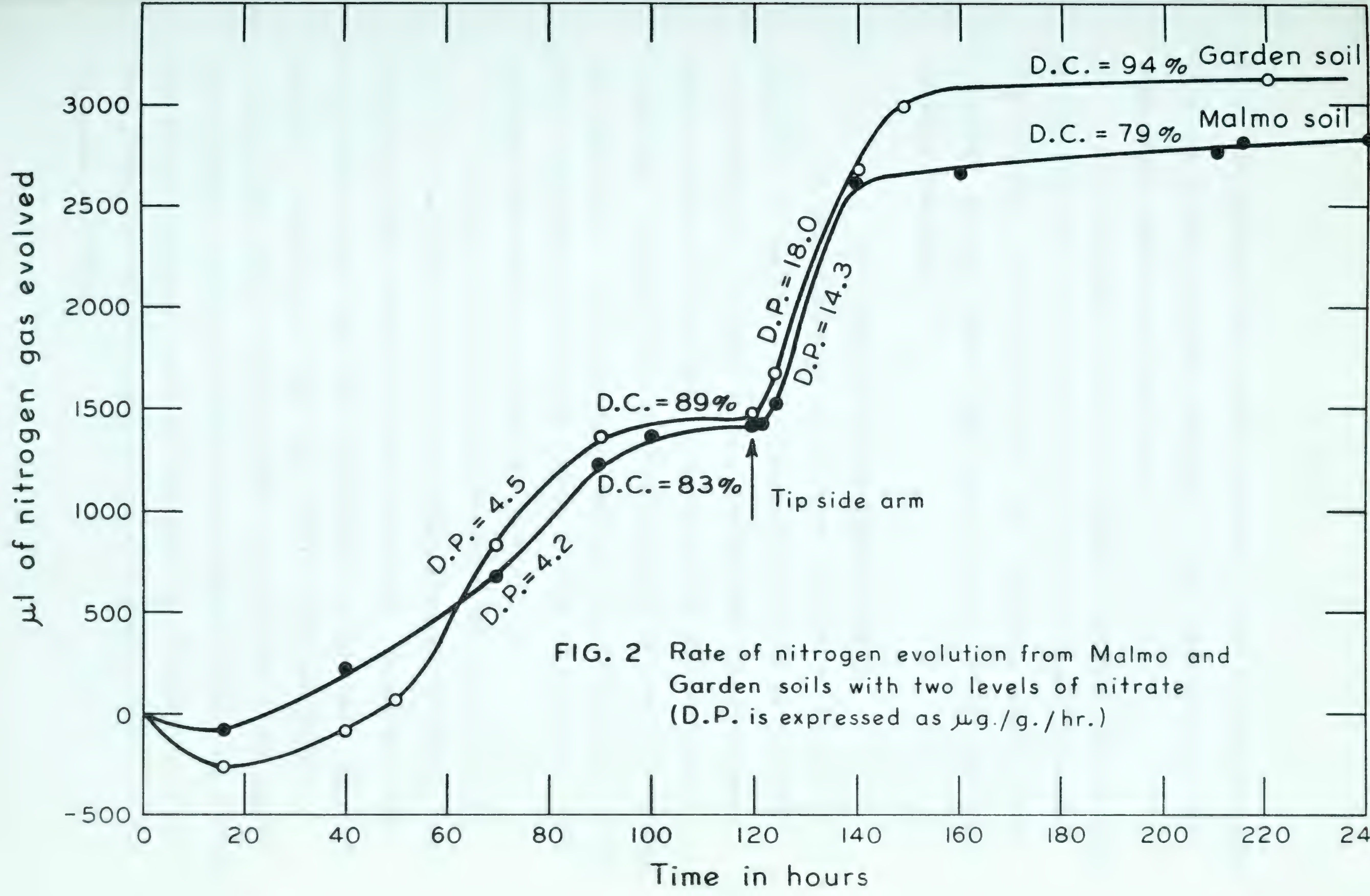

FIG. 2 Rate of nitrogen evolution from Malmo and Garden soils with two levels of nitrate (D.P. is expressed as µg./g./hr.)

other nitrogenous gases except nitrogen. Thus it was concluded that the product of denitrification in the conditions of the experiment was nitrogen gas alone.

On the basis of these preliminary results, the Warburg method for determining "Denitrifying Potential and Capacity" was adopted. It was decided that for each soil sample, three 'treated' and two 'check' runs should be carried out and the results averaged.

(c) Count of Denitrifiers in Soils

It was considered appropriate to examine the effect of anaerobic incubation of soils in Warburg flasks on the count of denitrifiers in these soils. It had been observed that there was a lag in the rate of evolution of nitrogen gas, especially in Malmo soil, and then the rate of evolution of gas was maintained. The recovery of added nitrate-nitrogen in these soils was found to be within 79 per cent to 89 per cent. The relevant information is given in Table IV.

The initial count of denitrifiers in Malmo soil was found to be remarkably low, presumably because the sample was a year old and stored in air dry condition. Anaerobic incubation has caused these few denitrifiers to multiply logarithmically. Garden soil which was freshly collected showed a considerable count of denitrifiers initially and there was no significant rise due to anaerobic incubation in Warburg flasks. The loss of nitrogen in both these soils is fairly close. It seems that the initial count had no significant effect on D.P. of these two soils.

TABLE IV

Effect of Anaerobic Incubation of Soil in the Presence of Nitrate on the Count of Denitrifiers

Soil	NO_3^--N Added (mg.)	D.C. %	Initial M.P.N.* Denitrifiers per g.	Final M.P.N.* per g. of Soil
Malmo	4.0	82	250	4.7×10^5
	2.0	79	250	3.6×10^4
Garden	4.0	89	$>10^4$	3.3×10^4
	2.0	79	$>10^4$	6.1×10^3

* M.P.N. = Most Probable Number

Nitrate-nitrogen was determined at the end of anaerobic incubation in Warburg flasks and none was found. Thus, it is apparent that some nitrate is converted to forms of nitrogen other than gas. In view of the low rise in population of denitrifiers in the anaerobic incubation of the two soils with nitrate, it is not likely that the proportion of nitrate not denitrified goes to form the bacterial protein of the denitrifiers. Since total counts of microorganisms could not be carried out, it may be possible that some other groups of microbial population might have assimilated this portion of nitrate for their nitrogen requirements.

RESULTS AND DISCUSSION

The study on volatile losses of nitrogen was carried out from two points of view, namely:

1. Do the soils of Alberta lose nitrogen as a gas under normal conditions, and if so, what effect do plants have on such losses?
2. Have the different soils of Alberta a potential for losing nitrogen as gas if suitable conditions, namely sufficient organic matter and anaerobic conditions, are provided?

For the first, greenhouse experiments were carried out, the details of which were outlined earlier. For the second, small samples (5.0 g.) were incubated in the Warburg flasks in the presence of nitrate and glucose.

(a) Greenhouse Experiments

Set No. I - Nitrogen Losses From Soils With Different Nitrogen Carriers

Soils treated with different carriers at uniform dose of 40 mg. N/100 g. plus one treatment without N, were kept moist in the greenhouse for 20 weeks without plants. Any young seedlings or grasses appearing were pulled out and buried in the soil. Since nitrogen can be lost from the soils in pots by leaching, plant uptake, and as volatile gases and since losses by the first two means were eliminated, any deficiency in total nitrogen is supposed to have occurred through volatilization. The data of losses are given in Table V. The

TABLE V

Changes in Nitrogen Content[1] of Some Alberta Soils Treated with 40 mg. N/100 g. as Different Nitrogen Carriers, and Kept Moist in Greenhouse Pots for 20 Weeks

Soil	pH	Initial[2] Total N Content (dry soil) mg./100 g.	No N[2] Treatment mg./100 g.	Total Nitrogen[3] in Treated Pots Expressed as mg. N/100 g.					
				Ammonium Sulphate	Ammonium Nitrate	Sodium Nitrate	Ammonium Phosphate	Urea	Dried Blood
Grey Wooded SiL	7.0	157	162*	125**	153*	155	149**	152*	162*
Black (Chernozemic) SiC	6.5	688	681	673	689	679	685	675	677
Black (Solonetzic) SiCL	5.4	589	622**	614**	591	605**	611**	605**	601*
Brown L	7.7	195	191*	188**	184**	191*	191*	182**	199*
Dark Brown L	7.5	224	227	225	228	195**	215**	207**	218*
Dark Grey Wooded CL	6.3	392	376**	388**	383**	385**	399**	396**	395*
Peat	6.1	2218	2206	2250**	2226	2274**	2213	2188**	2163**

1. Values are averages of four replications.
2. 40 mg. N/100 g. has been added to make these values comparable to the rest of the values.
3. Some samples gave odd results so they were repeated and the values close to the average were taken as the correct values

Significance indicated based on difference from respective check.

** P 0.01
* P 0.05

(Sample statistical calculation is shown in Appendix D)

'check' referred to under 'Methods' (Page 41) appears here as "initial" total nitrogen content (dry soil)" because these pots did not receive any nitrogen nor were they moistened. To make the values of initial total nitrogen content (check) and 'No N' treatment pots comparable to other treatments, 40 mg. N/100 g. was added to the actual total nitrogen content value of these pots. The nitrogen content in other treatments is shown as such and expressed as mg. N/100 g. of soil.

There are obvious differences in the response of different soils and the soil x nitrogen carrier interaction was found to be highly significant. It is therefore not possible to draw a common conclusion with regard to nitrogen losses, hence each soil and nitrogen carrier combination has to be considered separately and independently.

An examination of the nitrogen content of the 'check' and 'No N' treatment in Table V clearly shows that simultaneous fixation of nitrogen and denitrification has probably occurred in different soils. Therefore, in this table the statistical differences of mean values of total nitrogen due to different treatments have been shown with respect to initial total nitrogen content in the dry soil by placing asterisks to indicate the significance at the 5 per cent and 1 per cent levels. Thus, we have considered the dry soil as the 'control' and the pots without any nitrogen, but kept moist, as a treatment. Under the greenhouse conditions

and with the added nitrogen, it is not improbable that nitrogen fixation may have taken place in the treated pots, although it is likely that the extent of fixation in treated pots was somewhat lower than the pots to which no nitrogen was added.

Keeping soils moist in greenhouse pots, without any addition of nitrogen, caused significant changes in the nitrogen content in some soils. For example, Black (Solonetzic) soil gained 22.8 mg. N/100 g. soil, equivalent to 342 mg. N per pot, whereas Dark Grey Wooded soil lost 15.5 mg. N/100 g. soil, equivalent to 232.5 mg. N per pot. Significant fixation of nitrogen was also found in the case of the Grey Wooded soil, while the Brown soil lost a significant amount of soil nitrogen. The rest of the soils did not show significant changes in the 'No N' treatment when compared with their initial N content.

Grey Wooded, Brown, Dark Brown and Peat soils lost a significant amount of added nitrogen. Quite high losses took place in the case of Peat. In view of the high nitrogen content, (2.26 per cent) in the Peat itself, the addition of 40 mg. N/100 g., which is equivalent to 1.8 per cent of the total nitrogen in the Peat, is too small to give a correct picture of nitrogen loss or gain in Peat soils. Among the rest of the soil-nitrogen combinations, Dark Brown, Black (Solonetzic) and Grey Wooded soils gave the highest significant losses with sodium nitrate, ammonium nitrate and ammonium sulphate respectively. Unfortunately, due to variations within the replications, Black (Chernozemic) soil did not give significant results, although as high as 20 per cent of the added N as ammonium sulphate was lost.

Texture seems to have had some influence on the loss of added nitrogen from these soils. In general, coarse-textured soils showed a higher loss of added N.

So far as the different nitrogen carriers are concerned, the urea treatment caused significant losses in all the soils under study except Dark Grey Wooded. The loss with urea in Black (Chernozemic) soil was not significant. Some soils preferentially lost added nitrogen when applied in ammonium forms, e.g. Grey Wooded and Brown soils. Nitrate forms were lost from Black (Solonetzic) and Dark Brown soils, but the latter did not show any loss with ammonium nitrate.

Some soil-nitrogen carrier combinations caused significant increases, such as all treatments with Dark Grey Wooded soil, when compared with 'No N' treatment. It may be mentioned that the 'No N' treatment of Dark Grey Wooded soil lost about 15.5 mg. N/100 g. soil; therefore, all the significant gains will become insignificant if this loss is taken out from these treatments. It is likely that addition of fertilizer nitrogen had some inhibitory effect on the loss of soil nitrogen in this soil.

Mechanical analysis and pH data are presented in Table VI. The pH was determined on the soil samples from all the replications and the variations within the replications were found to be between 0.1 to 0.2 units of pH, therefore, only the average values are indicated. It is clear that pH was lowered by all the treatments in all the soils. Even the 'No N' treatment lowered the soil pH in most of the soils, although by not more than half a unit of pH. The treatment with sodium nitrate

TABLE VI

Effect on pH of Keeping Soils in Greenhouse Pots Moist with Different Nitrogen Carriers

Added at the Rate of 40 mg. N/100 g.

Soil	Texture				Initial Total N mg./100 g.	Initial pH	Final pH of the Soil After 20 Weeks Moist Incubation with Following Treatments						
	Sand %	Silt %	Clay %	Class			No N	Ammonium Sulphate	Ammonium Nitrate	Sodium Nitrate	Ammonium Phosphate	Urea	Dried Blood
Grey Wooded	28	48	24	SiL	157	7.0	6.7	4.8	5.3	6.5	4.9	5.4	5.5
Black (Chernozemic)	16	34	50	SiC	688	6.5	6.1	5.0	5.4	5.8	5.1	5.2	5.3
Black (Solonetzic)	22	50	28	SiCL	599	5.4	5.1	4.3	4.5	4.8	4.3	4.3	4.6
Brown	42	32	26	L	195	7.7	7.8	7.4	7.4	7.6	7.0	7.3	7.4
Dark Brown	50	32	18	L	224	7.5	7.4	5.6	6.2	7.0	5.4	6.2	6.4
Dark Grey Wooded	26	44	30	CL	392	6.3	5.8	4.9	5.0	5.6	4.7	5.1	5.0
Peat	-	-	-	-	2258	6.1	5.9	5.3	5.6	5.8	5.4	5.1	5.0

caused a lowering ranging from 0.1 to 0.7 unit of pH. The rest of the treatments caused a considerable lowering except in the case of the Brown soil. Hiltbold and Adams (1960) reported their results on the relationship of nitrogen volatilization to soil acidity resulting from nitrification and denitrification of added sources of nitrogen. They argued that gaseous loss of nitrifiable nitrogen precludes its equivalent acidity development, whether the loss occurs as ammonia prior to nitrification or as nitrogen gases during nitrification, whereas denitrification results in destruction of nitrate ion, leaving an equivalent alkaline residue regardless of gaseous products lost. They, therefore, surmised on correct theoretical considerations that deficiency in acidity should closely correlate to nitrogen volatilization. However, if nitrification is not appreciable, they predicted large increases in pH from denitrification. Their experimental results enabled them to successfully account for the differences between the calculated and actual acidity on the basis of nitrogen volatilization. Although it is difficult to translate the result of laboratory incubation tests to what may occur in the pots in the greenhouse, much less in the field, these conclusions of Hiltbold and Adams may help us to interpret some of the N losses and pH changes. It is most likely that the general decrease in pH in the greenhouse experiment was caused by nitrification. This is confirmed by the fact that in general, the sodium nitrate treatment is higher in pH than the rest of the treatments. The Brown soil (pH 7.7) has behaved quite differently from the others and the final pH in all treatments is maintained above pH 7.0. Since in this

soil, a significant loss of added N was observed with ammonium sulphate, ammonium nitrate and urea, it is highly probable that nitrification was accompanied by denitrification. The loss of 3.9 mg. N/100 g. soil in the case of 'No N' treatment accompanied by a slight rise in pH also shows that nitrification was accompanied by denitrification in this soil. If we follow this reasoning further in the case of other soils, it may be claimed that volatilization of nitrogenous gases during nitrification through chemical interactions or ammonia volatilization might have occurred, at least in those treatments where pH is markedly lowered. Greenland (1962) was of the opinion that serious loss of nitrogen in cultivated soil through denitrification is unlikely. The observation that nitrate is lost from field soils must therefore be, according to him, attributed to assimilation or to a chemical reaction leading to nitrate or nitrite removal. Similar opinions have been expressed by other workers (e.g. Clark *et al.*, 1960) suggesting that at low pH values, which may be caused by nitrification, nitrogen loss may be mainly due to breakdown of nitrous acid.

It is too much to claim complete validity on nitrogen loss data when the added 40 mg. N/100 g. ranges from 1.8 per cent of total N in Peat to 25.4 per cent of total N in Grey Wooded soil. The situation is further aggravated by nitrogen fixation in some soils and denitrification in some others. It is, therefore, considered that labelled nitrogen can help us to strengthen further any conclusions regarding the mode of loss of nitrogen from different soils.

Set No. II - Effect of Plants on Volatile Nitrogen Loss

For the second greenhouse experiment, Grey Wooded and Black (Chernozemic) soils were selected. The nitrogen carriers selected were ammonium sulphate, sodium nitrate and urea and added at the rate of 40 mg. N/100 g. of soil. These selections were based on the findings of previous greenhouse results. Although the Black (Chernozemic) soil did not give a significant loss in the first greenhouse experiment, this soil was used to check whether or not plants would affect denitrification loss. It was observed that mosses appeared on some of the pots towards the latter period of the experiment, as a result of excessive moisture. Pots of both soil types carried growth of these microscopic flora. However, in an earlier experiment with other soils in the greenhouse, no enrichment or deficiency in total nitrogen was found due to the presence of this microflora.

The results are presented in Tables VII and VIII. The values of nitrogen losses in pots with plants (barley) were arrived at as follows: Total dry weight in grams of plant material from each pot was multiplied by percentage of total nitrogen in plant material times 10 to give the mg. of nitrogen taken up by the plant from that pot. This value was then used to calculate the mg. of N/100 g. of soil for each pot and then added to the total nitrogen content of the soil which was also expressed as mg. of N/100 g. of soil. This final value can be subtracted from the 'No N' without plant treatment and expressed as a percentage of the added N (i.e. 40 mg. N/100 g. of soil) lost or gained as the case may be.

TABLE VII

Effect of Plants on Volatile Losses of Nitrogen and pH of the Grey Wooded Soil

	Initial N Content of soil mg./100 g.	N Content of 'No N' treatment mg./100 g.	Per Cent[1] Differences in Total N	Statistical[2] Significance	pH Values
Initial	138.3				6.8
TREATMENTS					
A. Without Plants					
No N		138.2		a	6.6
Ammonium Sulphate			-20	b	5.0
Urea			-20	b	5.5
Sodium Nitrate			- 7	c	6.3
B. With Plants[3]					
No N		139.7		a	6.8
Ammonium Sulphate			-11	c	5.2
Urea			-17	b	6.5
Sodium Nitrate			-10	c	7.0

---

1. Differences in total N content from 'No N' without plant treatment, expressed as per cent of added nitrogen (i.e. 40 mg./100 g.)

2. Mean differences in Column 4 followed by letter 'a' are significantly different from those not followed by 'a'; those followed by 'b' are significantly different from those not having 'b'. These calculations are based on Duncan's Multiple Range Test (Steel and Torrie, 1960).

3. Barley was seeded at the rate of 10 seeds per pot.

TABLE VIII

Effect of Plants on Volatile Losses or Gain of Nitrogen and pH of the Black (Chernozemic) Soil

	Initial N Content of soil mg./100 g.	N Content of 'No N' treatment mg./100 g.	Per Cent[1] Differences in Total N	Statistical[2] Significance	pH Values
Initial	620.0				6.4
TREATMENTS					
A. Without Plants					
No N		613.9		a	6.0
Ammonium Sulphate			- 1	a	5.1
Urea			+ 4	a	5.5
Sodium Nitrate			+34	b	6.0
B. With Plants[3]					
No N		613.7		a	6.3
Ammonium Sulphate			- 7	a	5.3
Urea			+ 6	a	5.8
Sodium Nitrate			+28	b	6.3

1. Differences in total N content from 'No N' without plant treatment, expressed as per cent of added nitrogen (i.e. 40 mg./100 g.)

2. Mean differences in Column 4 followed by letter 'a' are significantly different from those not followed by 'a'; those followed by 'b' are significantly different from those not having 'b'. These calculations are based on Duncan's Multiple Range Test (Steel and Torrie, 1960).

3. Barley was seeded at the rate of 10 seeds per pot.

There is one important difference between this experiment and Set No. I. While the first set was carried on for 20 weeks, this greenhouse experiment was carried on for only 7 weeks, because the barley plants were in the early heading stage and beyond this period the effect of the plants was considered of no consequence.

Subject to the above remarks, the losses of nitrogen without plants in Grey Wooded soil are comparable to earlier findings in Set I. The presence of plants resulted in more loss with sodium nitrate treatment. In the case of ammonium sulphate and urea, slightly smaller losses were observed when plants were present in the pots, although both were significant losses. Comparison of mean losses by Duncan's Multiple Range Test (Steel and Torrie, 1960) showed that in Grey Wooded soil, ammonium sulphate addition with and without plant treatments were significantly different, while urea and sodium nitrate did not show any difference between with and without plant treatments. It is therefore suggested that the presence of plants when ammonium sulphate is added as fertilizer will result in considerable conservation of nitrogen, which would have been otherwise lost. Losses observed in Black (Chernozemic) soil were not significant with any nitrogen carrier, both in the absence and presence of plants. Instead, significant gains with sodium nitrate treatments were found, which cannot be explained. Even the gains with sodium nitrate with and without plants are not significantly different. Thus, this experiment with Black soil for all intents and purposes is not meaningful so far as the presence of plants is concerned.

A long term experiment with high doses of nitrogen carriers or N^{15}, may result in a clearer picture of volatile losses of nitrogen in Black (Chernozemic) soil.

Plants accounted for 26 to 29 and 42 to 49 per cent of added nitrogen in Grey Wooded and Black soils respectively. In the pots with no added nitrogen, plants extracted 2.6 and 4.5 mg. N/100 g. of soil from these soils. It cannot, therefore, be claimed that the plant itself had any preferential effect on any soil-nitrogen carrier combination. It is of interest to mention the work of Woldendorp (1963) who attempted to put plants in truly denitrifying soil. He used artificial soil in an inert atmosphere and studied the effect of plants on the process of bacterial denitrification in soil and found that the presence of plants caused considerable losses of added nitrate. He found that the substances excreted by the roots served as hydrogen donors during denitrification. The greenhouse experiments were, however, drastically different in that we were measuring volatile losses of nitrogen (not necessarily through denitrification alone) under more or less normal conditions.

(b) Laboratory Studies on Denitrification

Similar treatments in the laboratory were used for all soil samples, namely two 'check' and three 'treated' Warburg flasks for each sample. The data obtained were expressed as average values. Standard deviations are shown only in the case of volume of nitrogen evolved to give an idea of variations in the results of that measurement.

1. Denitrification in Cultivated Surface Samples from Different Soil Zones of Alberta

Following preliminary experiments, the Warburg Method was tested on the seven surface samples from cultivated fields, described in Appendix A. The results obtained are presented in Table IX. The data include pH values, μl. of nitrogen gas evolved when 2 mg. of NO_3^--N was added to 5 g. of soil, and denitrifying capacity and potential values. It may be restated that D.C. and D.P. represent the inherent ability of the soil to convert the added nitrate to nitrogen gas, D.C. referring to the total amount produced and D.P. to the rate of production. These, under our experimental conditions, were dependent on the number of denitrifiers and the chemical and physical environment provided by the soil, and were, on the other hand, independent of the presence of nitrate, energy material and anaerobic conditions, because these were being provided.

These results show that the D.C.'s of these soils vary from 65 in Peat to 94 in the case of Dark Grey Wooded soil, and the D.P.'s vary from 1.37 in Peat to 3.44 in Black (Chernozemic) soil. Thus, Peat has the lowest denitrifying capacity and potential as compared to other soils. These results point out that under most favorable conditions, the soils under study can lose quite a fair amount of nitrate nitrogen present. Since adequate energy material (glucose) was also added, the situation represents conditions at its worst so far as nitrogenous fertilizer loss is concerned.

The rate of denitrification (i.e. D.P.) is fairly uni-

TABLE IX

Denitrification in Surface Soils of Cultivated Fields from Different Soil Zones of Alberta

Site No.	Soil Type	pH	µl. of Gas Evolved[1]	Denitrifying Capacity (D.C.) %	Denitrifying Potential (D.P.) %
1	Grey Wooded	7.0	1403 ± 31	79	2.27
2	Black (Chernozemic)	6.5	1330 ± 54	75	3.44
3	Black (Solonetzic)	5.4	1355 ± 30	76	2.55
4	Brown	7.7	1590 ± 66	89	3.04
5	Dark Brown	7.5	1610 ± 59	91	2.98
6	Dark Grey Wooded	6.3	1675 ± 82	94	2.45
7	Peat	6.1	1162 ± 29	65	1.37

[1] This value represents the average volume of nitrogen gas evolved after no further increase was observed and was calculated after deducting the average volume in the 'check' flasks from the average value of 'treated' flasks. The average figures are followed by Standard deviations.

Note: The lag period observed in all cases varied between 10-15 hours.

form in all soil samples except in the case of Peat. That low pH may be the cause of low denitrification in Peat is not borne out by comparatively high D.P. in the case of Black (Chernozemic) soil having a pH of 6.5 and also fairly high D.P. in the case of Black (Solonetzic) soil having a pH of 5.4. It is probable that in Peat, the number of denitrifiers are low and other chemical conditions provided by the substrate, e.g. growth promoting factors, micronutrients, etc. are not favorable. In the other soil samples, a high D.C. is not generally accompanied by a high D.P. On the basis of our results, it is not possible to put forward any plausible explanation. It is possible that interaction of such factors, as population of denitrifiers, total bacterial population, soil chemical and physical environment, etc., may be simultaneously affecting the situation. For example, the Black soils, Chernozemic and Solonetzic, have almost identical D.C. values, but the rate of nitrogen evolution was higher in Chernozemic than in Solonetzic.

When we compare these results with greenhouse experiment Set No. I with the same soils, some interesting observations can be made. The denitrification parameters obtained on the basis of the Warburg Method cannot be directly correlated to volatile losses of nitrogen under greenhouse conditions, which is rather obvious, especially because of anaerobic conditions in the Warburg flasks. Another reason for lack of correlation may be the oxidizable carbon (energy material for the bacterial population) in the normal soil. Moreover, under greenhouse conditions, the possibility of nitrogen fixation is always present, while this possibility is almost nil in the

Warburg flasks. Thus, the controlled condition provided in the laboratory for these soils, gives us information which can be applied in normal conditions only with great caution. The Brown and Dark Brown soils showed a high loss in the laboratory tests as well as with most of the nitrogen carriers in greenhouse experiments, suggesting that the major volatile loss of added nitrogen may be via denitrification. It is thus clear that the results obtained with the Warburg Method represent the potentiality of nitrogen loss by denitrification and may not correctly reflect the situation likely to occur under the natural greenhouse or field conditions because volatile losses can take place through other mechanisms as well.

2. Denitrification in Genetic Horizons of Different Soils of Alberta

The 36 samples collected from the different genetic horizons of the seven profiles in April 1964 were used for this study. These samples could not be used fresh in view of the slow turnover of samples on the Warburg apparatus. The results obtained, including details about the horizons, depth, pH and approximate lag period observed in the denitrification of the added nitrate, are presented in Table X. In order to appreciate the different rates of denitrification in different horizons of a single soil, the data for the Brown soil are plotted in Fig. 3. The lag period perhaps represents the time required for the denitrifiers to build up a population that can effectively carry on the process under the conditions of the experiment. It is also likely that some type of denitri-

TABLE X

Denitrification in the Soil Samples From the Genetic Horizons of Seven Different Profiles of Alberta Soils

Site No.	Soil Type	Horizon	Depth in.	pH	Lag Period Hours	Gas Evolved µl.	D.C. %	D.P. µg. N/g./hr.
1.	Grey Wooded	LH	0-1½	6.8	10-15	1732 ± 132	98	9.1
		Ae	1½-3½	7.0	10-25	1291 ± 87	73	1.8
		Bt_1	3½-12	6.8	30-40	522 ± 398	30	0.6
		Bt_2	12-24	6.8	30-40	215 ± 71	12	0.5
		Ck	24-36	7.9	30-50	62 ± 43	3	0.1
		C	36-45	8.4	30-50	202 ± 51	11	0.4
2.	Black (Chernozemic)	LH	0-1	6.8	3-5	1739 ± 421	98	5.25
		Ah	1-13	6.4	3-5	1845 ± 180	104	4.73
		Bm	13-20	8.3	35-40	976 ± 39	55	2.79
		Ck	20-29	8.5	40-80	509 ± 402	29	1.01
		C	29-42	8.4	50-60	200 ± 42	11	0.48
3.	Black (Solonetzic)	LH	0-2½	5.9	2-3	1453 ± 69	82	4.13
		Ah	2½-9	7.3	25-30	1753 ± 89	99	5.47
		Ae	9-14	8.2	20-30	1433 ± 185	81	2.90
		Bnt	14-20	8.5	50-60	414 ± 131	23	0.50
		Csk	20-27	8.7	-	3 ± 1	<1	0.0
		C	27-45	8.7	-	6 ± 5	<1	0.01

TABLE X

(Continued)

Site No.	Soil Type	Horizon	Depth in.	pH	Lag Period Hours	Gas Evolved µl.	D.C. %	D.P. µg. N/g./hr.
4.	Brown	Ah	0-7	7.9	15-20	1852 ± 17	104	7.8
		Bm	7-16	8.1	15-20	1623 ± 127	91	3.3
		Ck	16-24	8.3	20-22	1784 ± 74	100	5.4
		C	24-36	8.2	50-60	503 ± 84	28	1.3
5.	Dark Brown	Ah	0-8	7.2	5-10	1640 ± 6	92	5.39
		Ae	8-10	7.7	10-15	1284 ± 98	72	2.29
		Bt	10-16	8.0	5-10	1575 ± 95	89	5.35
		Ck	16-24	8.6	15-20	1368 ± 133	77	7.11
		C	24-36	8.8	20-25	479 ± 161	27	1.66
			36-43	9.0	50-70	1606 ± 377	91	2.33
6.	Dark Grey Wooded	Ah	0-14	6.7	3-5	1675 ± 336	94	3.43
		Bt_1	14-22	6.4	110-125	473 ± 50	27	0.44
		Bt_2	22-30	7.1	85-90	276 ± 42	16	0.35
		BC	30-41	7.9	35-40	1093 ± 314	62	1.35
		Ck	41-48	8.2	130-150	61 ± 36	3	0.66
7.	Peat	Organic layer (0-30 in.)	0-10	6.2	20-25	1483 ± 199	84	3.85
			10-20	7.7	90-95	1486 ± 303	84	6.19
			20-30	7.1	40-60	1607 ± 127	91	3.90
		Underlying material	30-40	6.8	30-50	1494 ± 276	84	11.29

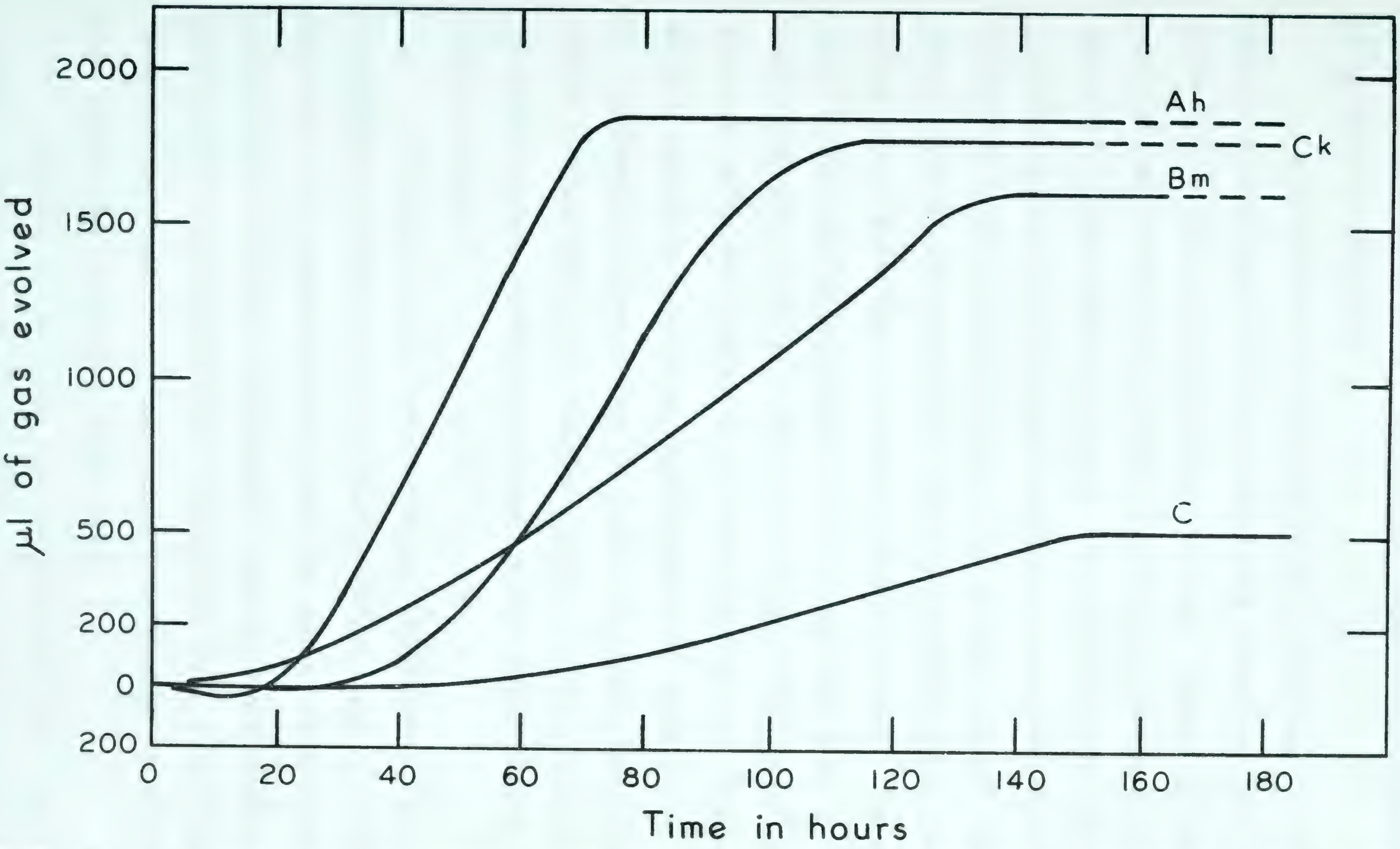

FIG. 3 Nitrogen volatilization from profile samples of Brown Chernozemic Soil when 5g. soil incubated with 2 mg. NO_3^- - N and glucose under complete anaerobic condition in Warburg flasks. Ah, Bm, Ck and C are different horizons (Table X) (results represent difference between averages of 3 treated and 2 check flasks)

[illegible]

fiers required this lag period for adaptation from aerobic to anaerobic metabolism.

One very clear trend generally observed in almost all soils, except Peat, is the decreasing values of D.C. and D.P. from the surface down to the C horizon. McGarity (1961) in a similar experiment using Black earth and Red-brown earth of Australia, but using no organic matter (glucose), thus making the denitrification process completely dependent on the content of native soil organic matter as an energy supply substrate, found that denitrification in the genetic horizons of these soils was a function of organic carbon content. In our experiment, since the energy material has been provided for the microorganisms over and above what might have been present in the soil samples, the gradual lowering capacity and potential, in all except a few cases, indicates that some other chemical or biochemical factors may be absent in lower horizons of these soils. Therefore, any further comment in this regard will have to await further experimentation to know what factor or factors are involved.

In some soils, such as Brown, Dark Brown, and Peat, considerable denitrification was observed even in lower horizons. It probably means that in these soils, other biological and chemical environments seem to be suitable for the process of denitrification. These data also show that denitrification is possible in the subsoil B-horizon of these soils even at depths of two to three feet.

The pH of the soil generally rises with depth in the profiles studied. The observation that the values of D.C. and

D.P. decrease as we go down the profile does not necessarily mean that higher pH is causing a slowing down of the process of denitrification. It is considered that other factors, such as the number of denitrifiers, growth factors, C:N ratio, micronutrients, etc. may be limiting the process rather than pH so far as the samples from the lower horizons of these soils are concerned. For example, in the Dark Brown soil, pH rose gradually from Ah through Ae to Bt, but the D.C. of Ah was 92, while of Ae it was 72 and again it increased to 89 in the Bt. The trend of D.P. was similar in these samples. Then, in the Ck which had a pH of 8.6, while the D.C. was only 77, the D.P. was 7.11, the highest value for this profile. The Ck showed a lag period of 15-20 hours. A somewhat similar situation can be observed if we examine the data obtained in the case of Brown soil.

A comparison of the data of surface samples of these soils, which were collected from virgin lands, with the data in Table IX for the samples in the previous section, which were collected from cultivated fields (0-6 in. depth), shows that generally the surface samples of virgin land have given higher values of both D.C. and D.P. than have the corresponding samples from cultivated fields. The respective samples are not significantly different in the pH values; thus it is likely that some other factor which is affected by cultivation may be involved in lowering the D.C. and D.P. of these soils.

3. Denitrification Study of Soil Samples of Rotation Plots for Different Years from Different Parts of Alberta

Samples from different plots of rotation trials obtained

from the Agricultural Research Station, Lethbridge, and the Experimental Farms at Lacombe and Beaverlodge, were used in this part of the study. The results obtained are presented in Tables XI to XIV. No specific differences in the lag period in the evolution of nitrogen gas in the Warburg flasks with these samples were observed, therefore, data for the lag period are not reported. Details of rotation, fertilizer treatments, as well as years of collection of samples, are also shown in these tables. These samples were collected without avoiding contamination and therefore from a microbiological point of view, they are considered to be uniform, particularly with regard to the number of denitrifiers within the samples from the same source. Further contamination, after these samples were brought into the laboratory, was avoided.

In Table XI, results for samples from the A-B-C Dryland plots, Lethbridge, for 1953 and 1963, are presented. The pH of these samples varies from 7.4 to 8.4 and the D.C. of these samples varies from 81 to 97 while the D.P. varies from 3.58 to 10.99. There are apparently no effects from the storing of samples for ten years on the rate of denitrification in these samples, although the samples from the same plot are not identical. This may be due to differences in rotation crops prior to sampling of the soil. Even soil samples from plots A, B-1 and B-2, which were similar in this respect, do not show any uniform effect. These soils do not differ very much in pH, although the 1963 samples are slightly higher in pH than the 1953 samples. It is not possible to find any general relationship of pH of the soils to their D.C. and D.P. values. In the case of soil from

TABLE XI

Denitrification in Soil Samples From A-B-C Dryland Plot, Research Station, Lethbridge (Soil - Dark Brown Solod, L to SiL on alluvial lacustrine)

Plot No.	Soil Sampling Following the Crop[1]	Year	pH	Gas Evolved µl.	D.C. %	D.P. µg./g./hr.
A	Wheat	1953[2]	7.5	1546 ± 117	87	4.85
	Wheat	1963	8.0	1488 ± 101	84	6.89
B-1	Fallow	1953	7.4	1617 ± 82	91	4.59
	Fallow	1963	8.4	1455 ± 52	82	5.37
B-2	Wheat on Fallow	1953	7.5	1439 ± 112	81	6.20
	Wheat on Fallow	1963	8.0	1494 ± 102	84	10.99
C-1	Fallow	1953	7.6	1471 ± 213	83	8.53
	Wheat on Fallow	1963	7.8	1571 ± 49	88	3.58
C-2	Wheat on Fallow	1953	7.6	1625 ± 28	92	6.36
	Wheat on Stubble	1963	8.0	1717 ± 68	97	5.87
C-3	Wheat on Stubble	1953	7.5	1575 ± 35	89	6.12
	Fallow	1963	7.9	1514 ± 197	85	4.43

[1] Rotation followed:
A - Spring Wheat Continuous
B - Fallow - Spring Wheat
C - Spring Wheat - Fallow - Spring Wheat

[2] Samples for 1953 were obtained by sub-sampling from the stored soil samples.

TABLE XII

Denitrification in Soil Samples From Rotation Plots[1], Research Station Lethbridge (Soil - Dark Brown Solod, L to SiL, on alluvial lacustrine)

Plot No.	Soil Sampling Following[1]	Year	pH	Gas Evolved µl.	D.C.	D.P.
5-6-S	Wheat and alfalfa	1955[2]	8.0	1190 ± 30	67	8.32
		1963	7.8	1188 ± 119	67	8.72
4-7-S	Wheat after sugarbeets	1955	7.7	1206 ± 44	70	8.23
		1963	7.9	1703 ± 70	96	12.11
4-1-S	Sugarbeets (1st year)	1955	8.0	1595 ± 103	90	7.62
		1963	8.1	1534 ± 44	86	7.13
5-8-S	Sugarbeets (2nd year)	1955	7.8	1421 ± 95	80	8.61
		1963	7.8	1668 ± 47	94	6.63

[1] Eight year rotation in progress since 1929 (Russell and Dubetz, 1958).

[2] Samples for 1955 were obtained by sub-sampling from the stored soil samples.

Note: All the plots represented by the samples were 'check' plots and thus had received no fertilizer.

TABLE XIII

Denitrification in Soil Samples From Rotation Plots of Project 10.01.10 (Old 6.1.6),

Experimental Farm, Lacombe (Soil - Eluviated Black, SiL, on lacustrine)

Plot No.	Soil Sampling Following[1]	Year	pH	Gas Evolved µl.	D.C.	D.P.
39	Wheat	1955	7.2	1667 ± 63	94	2.45
	Fallow	1963	6.8	1759 ± 84	99	2.36
48	Wheat	1955	7.0	1549 ± 200	87	5.11
	Fallow	1963	7.4	1903 ± 136	107	9.84

---

[1] Uniform rotation in the whole field was followed, namely

1954-55	Wheat
1956	Fallow
1957-61	Wheat
1962-63	Fallow

Fertilizer application in the two plots was as follows; which were applied in 1955, 1958, 1959, 1960 and 1961:

Plot No.	Before 1961	After 1961
39	33-0-0 @ 150 lb. per acre	27-14-0 @ 150 lb. per acre
48	33-0-0 @ 150 lb. per acre + 0-38-0 @ 52 lb. per acre	27-14-0 @ 150 lb. per acre

TABLE XIV

Denitrification in Soil Samples From Rotation Plots on Esher and Hythe Soil, Experimental Farm, Beaverlodge (Soil - Dark Grey Solod, CL, on lacustro-till)

Sample No.	Samples[1] From Plot	pH	Gas Evolved µl.	D.C.	D.P.
1-61	Check of Rotation[2] No. 1	5.9	1541 ± 49	87	3.29
4-61	M.F.[3] of Rotation No. 1	6.0	1570 ± 32	88	4.64
9-61	Check of Rotation No. 2	5.8	1397 ± 79	79	4.61
12-61	M.F. of Rotation No. 2	5.9	1438 ± 75	81	5.53
17-61	Check of Rotation No. 3	5.8	1990 ± 238	112	3.22
20-61	M.F. of Rotation No. 3	6.0	1419 ± 206	80	2.83
30-61	Rotation No. 4	5.8	1602 ± 30	90	3.44
36-61	Rotation No. 5	5.6	1689 ± 89	95	5.04

[1] These are composite samples collected in 1961.

[2] Rotations: No. 1 - Continuous Wheat
2 - Fallow - Wheat - Wheat
3 - Sweet Clover - Wheat - Wheat
4 - Fallow - Wheat - Hay - Hay - Oats - Oats
5 - Fallow - Wheat - Alfalfa - Alfalfa - Alfalfa - Oats

Samples were collected in late summer after the underlined crops in the respective rotation.

[3] M. stands for barnyard manure applied at 10 tons per acre.
F. stands for fertilizer (11-48-0) applied at 35 lb. per acre.

plot B-2, the 1963 sample with its higher pH shows a higher D.C. and D.P., but on the other hand, soil samples of the same year from plot C-3 with higher pH show lower D.C. and D.P. values, when they are compared with the corresponding samples for 1953. There is also no consistent variation due to rotation crops in these plots.

Table XII shows the effect of long term rotation on denitrification in the Dark Brown Solod soil from Lethbridge. These soil samples are not very different among themselves in pH, varying between 7.7 and 8.1. The results do not show any consistent difference in the rate of denitrification due to storage of samples. Among the rotation crops, wheat and alfalfa seem to have significantly decreased the D.C. values in both 1955 and 1963 samples, while the sugarbeet crop seems to have slightly increased the D.C. values. However, the D.P. does not seem to be affected by rotation practices or pH. Highest D.C. and D.P. values have been observed in 1963 samples from the wheat after sugarbeet plot.

Results of samples from rotation plots in Lacombe are presented in Table XIII. Due to some difficulties, samples from only two plots could be used for this study. These samples are similar in all respects except for the fertilizer treatments received before 1961. There are some differences in the denitrification parameters between 1955 and 1963 samples, and these differences are more pronounced in the samples from plot No. 48, which had received phosphorus.

For the samples from the rotation plots at Beaverlodge, obtained in 1961, the results obtained are presented in Table

XIV. These samples are also quite uniform in their pH values which vary between 5.6 and 6.0. The rotation and fertilizer treatments in the different plots are described in the table. With the exception of the check-plot with rotation No. 3, rotations No. 4 and No. 5 show higher D.C. values, while rotation No. 2 check shows the lowest D.C. values. On the other hand, D.P. values do not appear to be related to any differences in agronomic or other treatments of the plots. The D.C. of sample No. 17-61, namely check-plot with rotation No. 3, is the highest, although 3.22, the D.P. for this sample is relatively low. Such a high D.C. value warranted repetition of the test, but this could not be done because of the small sample available.

Considering all these samples from different parts of Alberta, representing Dark Brown Solod, Eluviated Black and Dark Grey Solod soils, and also differing considerably in texture and pH, the results obtained do not permit any definite relationship of rotation, pH, or any other characteristics of the soils to the denitrification parameters to be made. However, in a very general way, Dark Grey Solod with heavy texture and low pH from Beaverlodge has given low D.C. and D.P. values. It is, however, considered that the number of samples representing different rotations are not large enough to give significant differences due to rotation or other characteristics of the soil. On the same ground, it is not possible to appreciate the differences in D.C. and D.P. due to storage of samples on the basis of the results presented.

CONCLUSIONS

The first objective of the present study was to find out if nitrogen, added in different forms to the different soils of Alberta and kept in the greenhouse, is lost; and in those soils where such losses occur, whether the plant does or does not affect this loss. For this purpose, six soil samples from five of the soil zones of Alberta were used, namely Grey Wooded, Black, Brown, Dark Brown and Dark Grey Wooded, and in addition a sample of an Organic soil (Peat) was included. All were treated with six nitrogen carriers, namely ammonium sulphate, ammonium nitrate, sodium nitrate, ammonium phosphate, urea and dried blood. For the effect of plants on such losses, only two soils, namely Grey Wooded and Black (Chernozemic), were selected and treated with ammonium sulphate, urea and sodium nitrate. Each combination of soil and nitrogen carrier had pots with and without plants. The nitrogen carrier in both these sets of greenhouse experiments was added at the rate of 40 mg. N/100 g. soil, which is equivalent to 800 lb. N per acre assuming that 6 inches of surface soil normally weigh two million pounds per acre. The second objective was to find out the potentiality of true bacterial denitrification in these soils that were used in the greenhouse and also in the genetic horizons of these soils from virgin land. This aspect was further expanded to find out if agronomic practices such as rotation, fertilizer treatments, etc. and storing of samples have effects on the process of denitrification in some soils of Alberta.

The results of the greenhouse experiment indicate that significant losses of added nitrogen took place with one or more of the nitrogen carriers in Grey Wooded, Black (Solonetzic), Brown and Dark Brown soils. In view of considerable variations within the replications and also because the added nitrogen was too low in proportion to the total nitrogen in Peat, the results for that soil are considered unreliable. Among the various nitrogen carriers, treatment with urea caused losses from almost all soils. Grey Wooded and Brown soils were found to preferentially lose nitrogen when applied in ammonium form. With nitrate, no such preference in any soil could be observed. The interaction of soil and nitrogen carriers was found to be highly significant implying that no blanket conclusion on the loss of nitrogen from these soils with any specific nitrogen carrier is possible.

Dark Grey Wooded soil with all nitrogen carriers and Brown soil with dried blood as nitrogen carrier showed significant increases in their nitrogen content when compared with their respective 'No N' treatments.

Soil pH determined at the end of the experiment showed marked although varying decreases with all treatments. This lowering of pH is due to the nitrification process going on in these soils. Denitrification, on the other hand, causes increase in pH. Thus the deficiency in acidity can be correlated to nitrogen volatilization (Hiltbold and Adams, 1960). In the soils used, discussion on these lines suggested that volatilization took place not only through the process of denitrification but also by way of chemical reactions involving products

of nitrification, such as nitrous acid.

Effects of plants on volatile losses of nitrogen were examined and it was found that the presence of plants in Grey Wooded soil reduced volatile losses when ammonium sulphate was the nitrogen carrier. In the case of urea and sodium nitrate, the presence of plants was found to have no significant effect.

The second objective, that of finding out the biochemical ability of a soil to support true bacterial denitrification was accomplished by using Warburg apparatus. The Warburg method has been adopted very recently for the study of denitrification. In the preliminary studies, it was found that with a known quantity of nitrate and glucose, not only the amount of nitrate denitrified but also the rate of nitrogen gas evolution fully describes the nature and extent of denitrification in a particular sample. Accordingly, two parameters, Denitrifying Capacity (D.C.) and Denitrifying Potential (D.P.), were proposed, defined and used to express the results. When 5 g. of sample was treated with 2 mg. NO_3^--N and 50 mg. C as glucose at near field moisture capacity and incubated under fully anaerobic conditions and the volume of nitrogen evolved was observed until no more increase in the volume of nitrogen evolution could be noted, "Denitrifying Capacity" was defined as the per cent of added NO_3^--N lost as gas and "Denitrifying Potential" as the µg. of N/g. of soil/hour.

With the same soil samples that were used in the greenhouse experiments, it was found that all have the potentiality to lose nitrogen from the added nitrate if anaerobic conditions

and energy materials are provided. Denitrifying parameters obtained by using this method, however, could not be correlated to nitrogen losses found under greenhouse conditions.

The study of denitrification in the genetic horizon samples, from the same place as the above mentioned surface samples, gave some interesting results. McGarity (1961) found that in samples from lower horizons, organic carbon content was the limiting factor for the denitrification process. In this study, sufficient organic matter as glucose was applied to the soil, yet in lower horizons of almost all the profiles, all the added nitrate was not denitrified. It is considered that some other factor or factors, e.g. number of denitrifiers, growth factors, C:N ratio, micronutrients, etc., or their interactions may be involved. These results, however, show that denitrification does take place in sub-soils of these soils, although the rate of the process is not very fast.

The surface samples from cultivated and virgin soils have different D.C. and D.P. values and in respective samples, soils from cultivated fields gave lower values. Thus it is possible that cultivation brings about certain changes in the environment of the soil which decreases the extent of denitrification in these soils.

The results of the soil samples from rotation plots obtained from different parts of Alberta did not allow for a definite conclusion so far as effect of rotation and fertilizer practices are concerned. Generally, rotation crops and fertilizer practices were found to have no significant effect on the rate of denitrification of these soils.

Study of stored samples, compared with fresh or recently obtained samples, gave no conclusive results, however, the number of samples was not enough to allow any general statement in this regard to be made.

On the basis of the experiments and interpretation of accumulated data, it may be assumed that soils of Alberta can lose significant amounts of fertilizer nitrogen. Furthermore, they have the potentiality of losing considerable amounts of nitrogen through the process of denitrification when suitable conditions occur. The lower horizons of all the soils studied showed some capability to lose nitrogen by denitrification. The factor limiting the process does not appear to be the lack of oxidizable carbon alone: it is suggested that lack of amino acids, vitamins, micronutrients, etc. may also be involved. The evaluation of nitrogen losses by the Kjeldahl method, as recommended by Bremner and Shaw, is considered to be an unsatisfactory procedure, at least for the soils used in this study.

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APPENDIX A

General Information About Soils Collected For Greenhouse and Profile Samples For Experiments From Different Soil Zones in Alberta

Site No.	Soil Zone	Address	Legal Location	Classification	Description*
1	Grey Wooded	South of Chipman	SE ¼-14-53-19 W of 4	Orthic Grey Wooded	Cultivated fire -break. Not cropped
2	Black (Solonetzic)	Namao	SW ¼-36-54-24 W of 4	Solodized Solonetz (Solonetzic Order)	Cultivated field
3	Black (Chernozemic)	Namao	SW ¼-12-55-24 W of 4	Orthic Black Chernozemic	Cultivated field
4	Brown	Vauxhall	SE ¼-16-14-16 W of 4	Orthic Brown Chernozemic	Cultivated field
5	Dark Brown	Vulcan	NW ¼-24-16-26 W of 4	Eluviated Dark Brown Chernozemic	Cultivated field
6	Dark Grey Wooded	Ardrossan	NE ¼-35-52-22 W of 4	Dark Grey Wooded (Podzolic Order)	Cultivated field
7	Peat	Foley Lake	NE ¼-12-51-23 W of 4	Organic Soil	Uncultivated, having cover of very thick grass.

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* Samples from profiles for Denitrification in Warburg flasks were collected from virgin land situated close to these fields.

APPENDIX B

Calculating Gas (Nitrogen) Evolution From Manometric Readings

(DATA SHEET)

Prep: Soil Sample pH: Temp: 30°C Date: April 10 '64
'Malmo'

Flask #		T.B.[a]		2					3				
In Cup				Soil without NO_3^--N					Soil with 2 mg. NO_3^--N and glucose				
Side 1				–					–				
Side 2/Well				.3 ml. of 10% KOH					Same as in #2				
K[b]				1.6938					1.7920				
Time	Int.	A	Δ	A	B	C	D	E	A	B	C	D	E
1:00[c]	-	102	-	247	-				251				
10:00	9	29	-73	125	-122	-49	-49	-83	136	-115	-42	-42	-88
9:00	20	-63	-165	58	-189	-24	-24	-40	78	-173	-8	-8	-14
9:00	20	126	-	58	-				78	-			
5:00	40	200	+74	179	+121	+47	+23	+39	250	+172	+98	+90	+161
5:00	40	200	-	179	-				80	-			
1:00	48	245	+45	233	+54	+9	+32	+54	167	+87	+42	+132	+237
1:00	48	245	-	65	-				90	-			

a. Thermal Barometer

b. K-value or flask constant is calculated from the following formulae:

$$\frac{V_g \frac{273}{T} + V_f \alpha}{P_o}$$

when
Vg - volume of gas phase in flask in ul.
Vf - volume of fluid in flask in µl.
α - solubility of gas in reaction liquid as ml./ml.
Po - Standard pressure, which is 10,000 mm. Kreb's fluid.
T - Temperature of bath in °A.

c. Instructions for recording as follows:

APPENDIX B

(Continued)

Instructions for Recording the Readings and Calculations

Readings can be recorded and calculated, either by "Total Method" or by "Interval Method". A modification of "Total Method" was adopted for our purpose, because after certain intervals, some gas had to be released from the Warburg flasks to continue the readings. The instructions for filling the columns on the "DATA SHEET" are as follows:

Time: Actual time reading is taken.

Interval: Interval in hours and minutes from the start of the Experiment.

Column A: The actual readings from the open arms of the manometers, including T.B., are recorded under this column in mm. These are the pressure readings when the manometer fluid in the closed arm of the manometer is adjusted to the reference point (150 mm. in our case).

Column Δ: The changes in pressure in the T.B. are entered here.

Column B: The changes in pressure, due to evolution of gas in the reaction vessel, are entered here. The values under this column are obtained by subtracting the initial reading from all the subsequent readings under the column until the open arm is not sufficient to give readings and gas is released from the flasks.

Column C: This is the corrected reading and is obtained by subtracting the value in Δ from B.

APPENDIX B

(Continued)

Column D: This is the accumulation total pressure from the start of the experiment.

Column E: The values in D are multiplied by K-value to obtain the µl. of gas evolved.

To calculate D.C. and D.P., the volume in µl. was multiplied by 1.127 to get µg.N. The factor 1.127 was arrived at as follows:

22.4 µl. of N_2 at S.T.P. = 1 µmole of N_2

or

24.86 µl. of N_2 at 30°C and 760 mm. Hg pressure = 1 µmole of N_2

$\therefore$ 1 µl. of N_2 " " = $\frac{1}{24.86}$ µmole

or

$\frac{28.016}{24.86}$ µg. of N_2

i.e. 1.127 µg. of N_2

APPENDIX C

STANDARD CURVE FOR NITRATE DETERMINATION

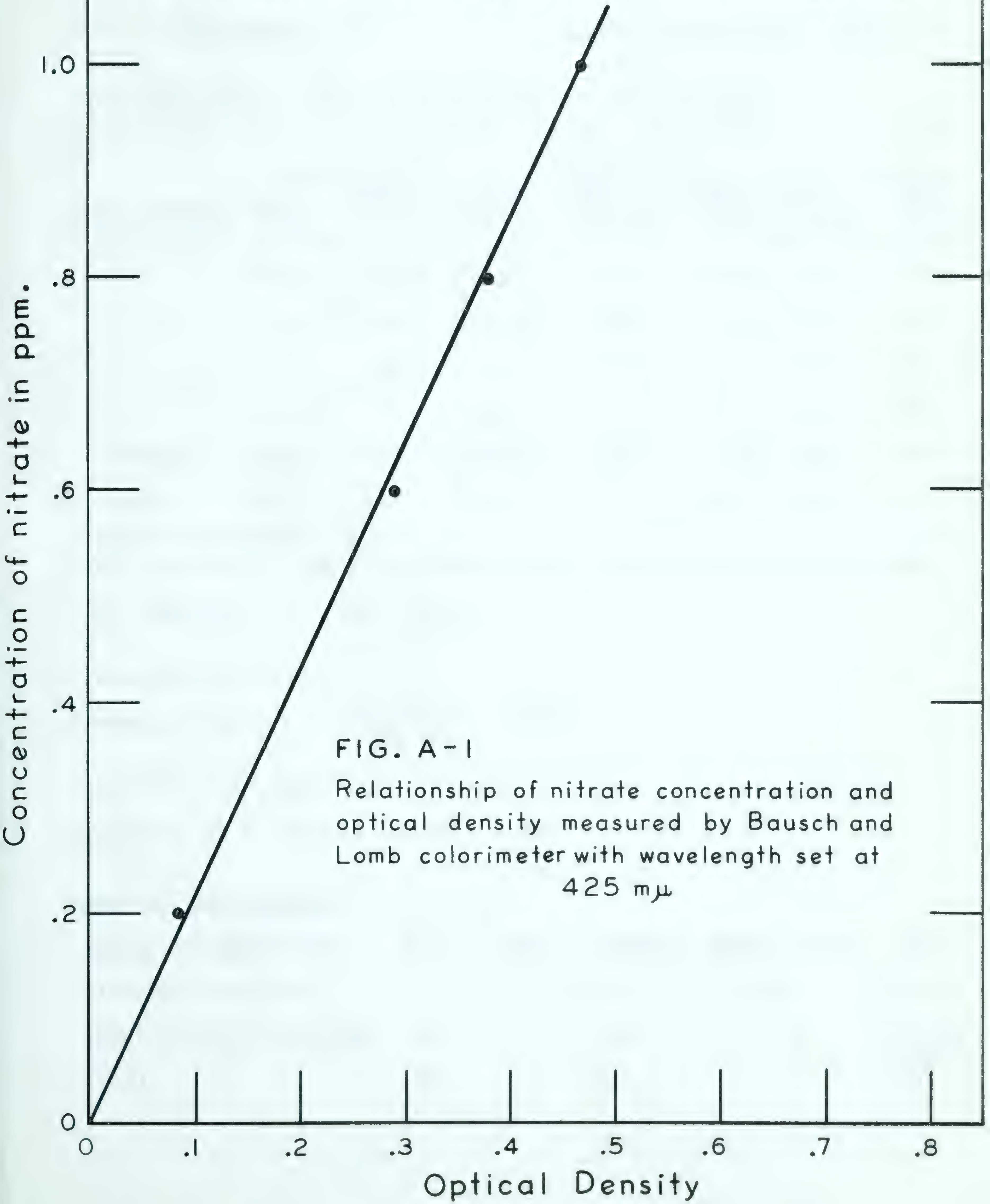

FIG. A-1

Relationship of nitrate concentration and optical density measured by Bausch and Lomb colorimeter with wavelength set at 425 mμ

APPENDIX D

Sample Statistical Analysis

Soil: Grey Wooded   Expt: Greenhouse - Set No. I

Total Nitrogen in each pot expressed as mg. N./100 g. soil

	Treatments						
Replications	No N[1]	Ammon. Sulph.	Ammon. Nitrate	Sodium Nitrate	Ammon. Phos.	Urea	Dried Blood
1	160.1	146.2	148.1	155.7	148.6	152.4	166.0
2	164.4	139.2	157.0	156.8	146.9	150.9	163.5
3	163.0	148.1	154.6	157.0	147.7	151.7	162.8
4	161.6	145.4	151.3	150.0	152.8	152.1	156.3
Total	649.1	579.3	611.0	619.5	596.0	607.1	648.6
Mean	162	145	153	155	149	151	162

[1] 40 mg. N/100 g. soil is added to the actual calculation to make it comparable to other treatments.

Grand total = 4310.6

Correction factor = $\frac{(4310.6)^2}{28}$ = 663,617

Total SS = 665,298 - c.f. = 1,681

N-carrier SS = 664,622 - c.f. = 1,005

Analysis of Variance

Source of Variation	D.F.	Sum of Squares	Mean Square	F
Nitrogen carriers	6	1,005	167.5	5.20**
Error and Replications	21	676	32.2	highly significant
Total	27	1,681		

APPENDIX D

(Continued)

$$S\bar{x} = \sqrt{\frac{32.2}{4}} = \sqrt{8.05} = 2.84$$

$\therefore$ Lsd. = $S\bar{x} \cdot \sqrt{2} \cdot t$ = 8.3 at 5% level, and 11.2 at 1% level.
i.e. 21 at 5% level, and 28 at 1% level,
when expressed as % of added N (i.e.
40 mg. N/100 g. soil).

Comparison of the Means:

Treatments	Mean mg./100 g.	Difference From 'No N'	Percent of Added N
No N	162		
Ammonium Sulphate	145	-17	-44**
Ammonium Nitrate	153	-9	-24*
Sodium Nitrate	155	-7	-19
Ammonium Phosphate	149	-13	-33**
Urea	151	-11	-26*
Dried Blood	162	0	0

from the Agricultural Research Station, Lethbridge, and the Experimental Farms at Lacombe and Beaverlodge, were used in this part of the study. The results obtained are presented in Tables XI to XIV. No specific differences in the lag period in the evolution of nitrogen gas in the Warburg flasks with these samples were observed, therefore, data for the lag period are not reported. Details of rotation, fertilizer treatments, as well as years of collection of samples, are also shown in these tables. These samples were collected without avoiding contamination and therefore from a microbiological point of view, they are considered to be uniform, particularly with regard to the number of denitrifiers within the samples from the same source. Further contamination, after these samples were brought into the laboratory, was avoided.

In Table XI, results for samples from the A-B-C Dryland plots, Lethbridge, for 1953 and 1963, are presented. The pH of these samples varies from 7.4 to 8.4 and the D.C. of these samples varies from 81 to 97 while the D.P. varies from 3.58 to 10.99. There are apparently no effects from the storing of samples for ten years on the rate of denitrification in these samples, although the samples from the same plot are not identical. This may be due to differences in rotation crops prior to sampling of the soil. Even soil samples from plots A, B-1 and B-2, which were similar in this respect, do not show any uniform effect. These soils do not differ very much in pH, although the 1963 samples are slightly higher in pH than the 1953 samples. It is not possible to find any general relationship of pH of the soils to their D.C. and D.P. values. In the case of soil from

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extra page

TABLE XII

Denitrification in Soil Samples From Rotation Plots[1], Research Station Lethbridge (Soil - Dark Brown Solod, L to SiL, on alluvial lacustrine)

Plot No.	Soil Sampling Following[1]	Year	pH	Gas Evolved µl.	D.C.	D.P.
5-6-S	Wheat and alfalfa	1955[2]	8.0	1190 ± 30	67	8.32
		1963	7.8	1188 ± 119	67	8.72
4-7-S	Wheat after sugarbeets	1955	7.7	1206 ± 44	70	8.23
		1963	7.9	1703 ± 70	96	12.11
4-1-S	Sugarbeets (1st year)	1955	8.0	1595 ± 103	90	7.62
		1963	8.1	1534 ± 44	86	7.13
5-8-S	Sugarbeets (2nd year)	1955	7.8	1421 ± 95	80	8.61
		1963	7.8	1668 ± 47	94	6.63

[1] Eight year rotation in progress since 1929 (Russell and Dubetz, 1958).

[2] Samples for 1955 were obtained by sub-sampling from the stored soil samples.

Note: All the plots represented by the samples were 'check' plots and thus had received no fertilizer.